CLINICAL STUDIES USING A HIGH SENSITIVITY SHADOW-SHIELD MOBILE WHOLE BODY MONITOR

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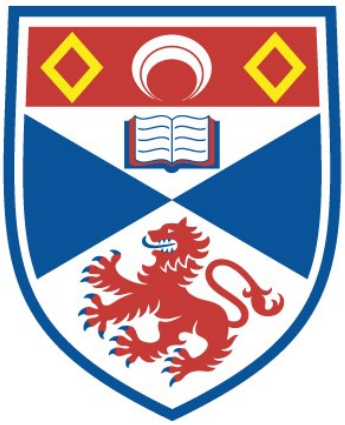

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THESIS

presented to

THE UNIVERSITY OF ST. ANDREWS

for the Degree of

DOCTOR OF PHILOSOPHY

by

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ABSTRACT

This thesis describes research conducted by the author using a high sensitivity, shadow-shield, mobile, whole body monitor and is primarily concerned with its clinical applications.

Whole body counting-rates vary when administered radioisotopes are redistributed in the body. Several methods of assessing the magnitude of geometrical variations in counting-rates have been brought together. Point sources in simple water phantoms, a life-like phantom man with simulated organs and human subjects were studied and the relative importance of the sources of variation and the value of the methods assessed.

Cobalamin excretion rates were studied in patients with renal and hepatic disease from about one week after injection when, evidently the tracer dose of vitamin B_{12} has effectively equilibrated with body stores. The daily cobalamin loss was abnormally high in 6 out of 8 patients with renal disease and 3 out of 6 with hepatic disease.

In evaluating the treatment of vitamin B_{12} deficiency, the retention of three parenterally administered vitamin B_{12} compounds was investigated. From the results, maintenance schedules could be derived empirically for patients with uncomplicated vitamin B_{12} deficiency and with concomitant renal or hepatic disease.

Using a double tracer technique, the oral absorption at different dose levels of coenzyme B_{12} and other cobalamins was measured. The fraction of the dose absorbed appears to be a function of both the mass

and the structure of the cobalamin.

The human prostate gland contains a high concentration of zinc and when a carcinomatous change occurs in the gland, there is a distinct fall in the zinc content of the prostatic tissue. A preliminary study of the whole body metabolism and local prostatic uptake of zinc-65 was undertaken to provide the basis of a method for measuring the response of prostatic carcinoma to therapy.

The metabolism of iron-59 was studied in an investigation of the anaemia in patients with chronic renal failure and in patients also receiving regular dialysis therapy. It was suggested that the anaemia results from the depression of erythropoiesis rather than iron deficiency. The high iron turnover rate in patients receiving regular dialysis could be explained almost entirely by the blood losses in the Kolff twin-coil artificial kidney. Methods of minimising these blood losses are suggested.

The monitor was calibrated using potassium-42 for the measurement of total body potassium. The precision of the calibration and monitoring procedures were examined in detail. A relationship was derived enabling a calibration factor to be calculated from a subject's weight and height. In clinical practice, the measurement of total body potassium may only be meaningful if the 'normal' value is known; this was correlated with the weight, height and age of the subject. To illustrate these techniques, total body potassium was measured in an investigation of the potassium status in patients with chronic renal failure, rheumatoid arthritis and following ureterosigmoid anastomoses.

PREFACE

This thesis, composed by the author, contains an account of research conducted by her at the Scottish Research Reactor Centre, East Kilbride. The analysis of the results and their interpretation are those of the author. The thesis has not been accepted in fulfilment of the requirements of any other degree or professional qualification.

Research was undertaken in clinical studies using a high sensitivity shadow-shield mobile whole body monitor. The author was admitted to the University of St. Andrews under Ordinance General No. 12 as a research student and as a candidate for the degree of Doctor of Philosophy in October, 1967.

STATEMENT

I certify that to the best of my knowledge the Thesis submitted by Miss Priscilla King has been prepared in conformity with the Ordinances and Regulations governing the award of the Degree of Doctor of Philosophy in the Faculty of Science of the University of St. Andrews.

Signed: Anthony E. Ritchie, MA, B.Sc, MD, FRSE.

Supervisor on behalf of the University of St. Andrews.

5th January, 1971

PUBLICATIONS

The following papers have been published or submitted for publication with which the present author has been associated:

1. BODDY, K. : The development of a prototype shadow-shield whole-body monitor. Phys. Med. Biol. 12, 43-50, 1967.
2. BODDY, K. : A high sensitivity shadow-shield whole body monitor with scanning-bed and tilting-chair geometries, incorporated in a mobile laboratory. Brit. J. Radiol. 40, 631-637, 1967.
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18. BODDY, K., KING, PRISCILLA C., TOTHILL, P. and STRONG, J.A.: Measurement of total body potassium with a high sensitivity shadow-shield whole body counter: calibration and sources of error. Phys. Med. Biol. In press.

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20. BODDY, K., KING, PRISCILLA C., HUME, R. and WEYERS, ELSPETH : Prediction of total body potassium from weight, height and age. Submitted for publication.

21. HUME, R., ROWAN, T., WEYERS, ELSPETH , BODDY, K. and KING, PRISCILLA C. : The relationship between red cell volume potassium and total body potassium and lean body mass. Submitted for publication.

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25. BODDY, K., KING, PRISCILLA C., BRIGGS, D., LINDSAY, R. and KENNEDY, A.C. : Total body potassium in uraemic patients and the study of potassium kinetics during dialysis. Submitted for publication.

26. LAWSON, D.H., BODDY, K., KING, PRISCILLA C., LINTON, A.L. and WILL, G. : Iron metabolism in patients with chronic renal failure. II. During regular dialysis treatment. Submitted for publication.

27. LAWSON, D.H., BODDY, K., KING, PRISCILLA C., LINTON, A.L. and WILL, G. : Iron metabolism in patients with chronic renal failure. III. With functioning renal homografts. In preparation.

28. BODDY, K., KING, PRISCILLA C., LAWSON, D.H., LINTON, A.L. and WILL, G. : Measurement of blood loss before and after renal transplantation. In preparation.

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CONTENTS

CHAPTER I

INTRODUCTION

The harnessing of nuclear power for the service of man, which may be regarded as the greatest scientific and technical achievement of the century, has been accompanied by the development of another new technology, different in essence, yet inseparable from it, the production and uses of radioactive isotopes. Artificially radioactive materials, made available through the use of nuclear reactors, have made valuable contributions, not only in the science laboratory, but also in such practical fields as medicine, agriculture and industry.

Nuclear medicine is that clinical discipline concerned with the diagnostic, therapeutic and investigative uses of radionuclides. The great advance which has taken place in this subject during the past decade would not have been possible without the rapid growth in nuclear science and technology during the same period. Radiation detectors and the associated electronic equipment have been greatly improved and new developments made leading to a whole generation of equipment some specifically for the needs of nuclear medicine, such as whole body counters, radioisotope scanners and scintillation cameras.

Whole body counting consists of the measurement of radioactivity

in the body by a sensitive external radiation - detecting instrument. It is therefore confined to the measurement of radioisotopes which emit radiation capable of penetrating the body tissues and other absorbing media positioned between the radioactive deposit in the body and the radiation - sensitive detector. The quantities of radioactive elements or labelled compounds in the body at the time of measurement can be assessed. Medical research workers were among the first to realise the potentialities of the radioactive tracer technique as a means of studying the complex metabolic processes which go on inside the human body. If periodic whole body measurements are combined with simultaneous measurements of the radioactivity in blood, tissue or organs, valuable information can be obtained concerning the metabolism of radioactive tracers. They therefore form the basis of a most powerful technique for extending medical knowledge. They have been especially useful in establishing the role of elements which are present only in very small quantities in the body, yet seem to be vital to its health.

The work described in this thesis has been carried out in the MERLIN * mobile shadow-shield whole body monitor. The mobile unit was developed since no hospital in Scotland possessed or had easy access to a high sensitivity counter equal to that of a steel or lead room. The Scottish Hospital Endowments

(*Monitoring Equipment for Radioactivity in Low levels IN vivo.)

Research Trust awarded a capital grant of £19,745 in May, 1965, for the construction of the mobile monitor (Boddy,1967). The exterior of the vehicle is shown in Fig. 1 and the interior in Fig. 2.

The shielding comprises 7 tons of premonitored virgin lead bricks of 2 inch thickness (Fig. 3). A sodium iodide detector, 29.2 cm diameter by 10.2 cm is housed in the central turret. The internal width of the monitor is 61 cm. A T.M.C. 400 channel pulse height analyser with a resolver integrator unit and a type-punch-read control unit including EHT supply were chosen. Other equipment includes typewriter, tape readout and a tape reader. Filter and stabiliser units are used to minimise fluctuations and surges in the mains voltage. To allow the change from the mains electrical supply to the vehicle generator, and vice versa, to be made without de-energising and therefore de-stabilising the electronic equipment, a change-over unit was installed. The lay-out of the electronic equipment is shown diagrammatically in Fig. 4.

The scanning-bed geometry is employed in which the patient, lying on a motorised couch, passes beneath the detector from head to feet in the supine position and in the reverse direction in the prone position. The scanning length can be varied up to 275 cm and measurements made with a bed to crystal face distance of 30 cm (low position) and 41 cm (high position). The speed

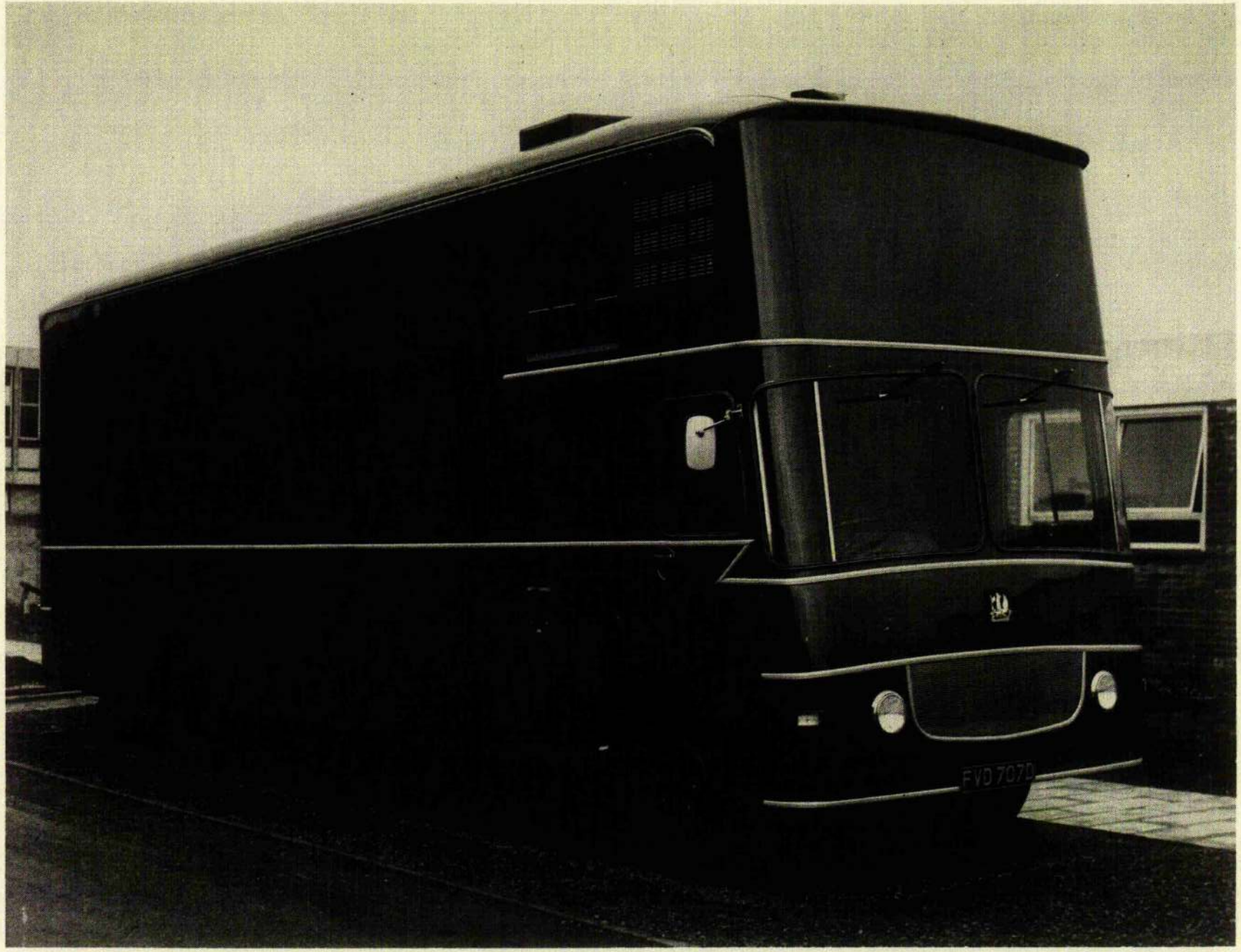


Fig. 1

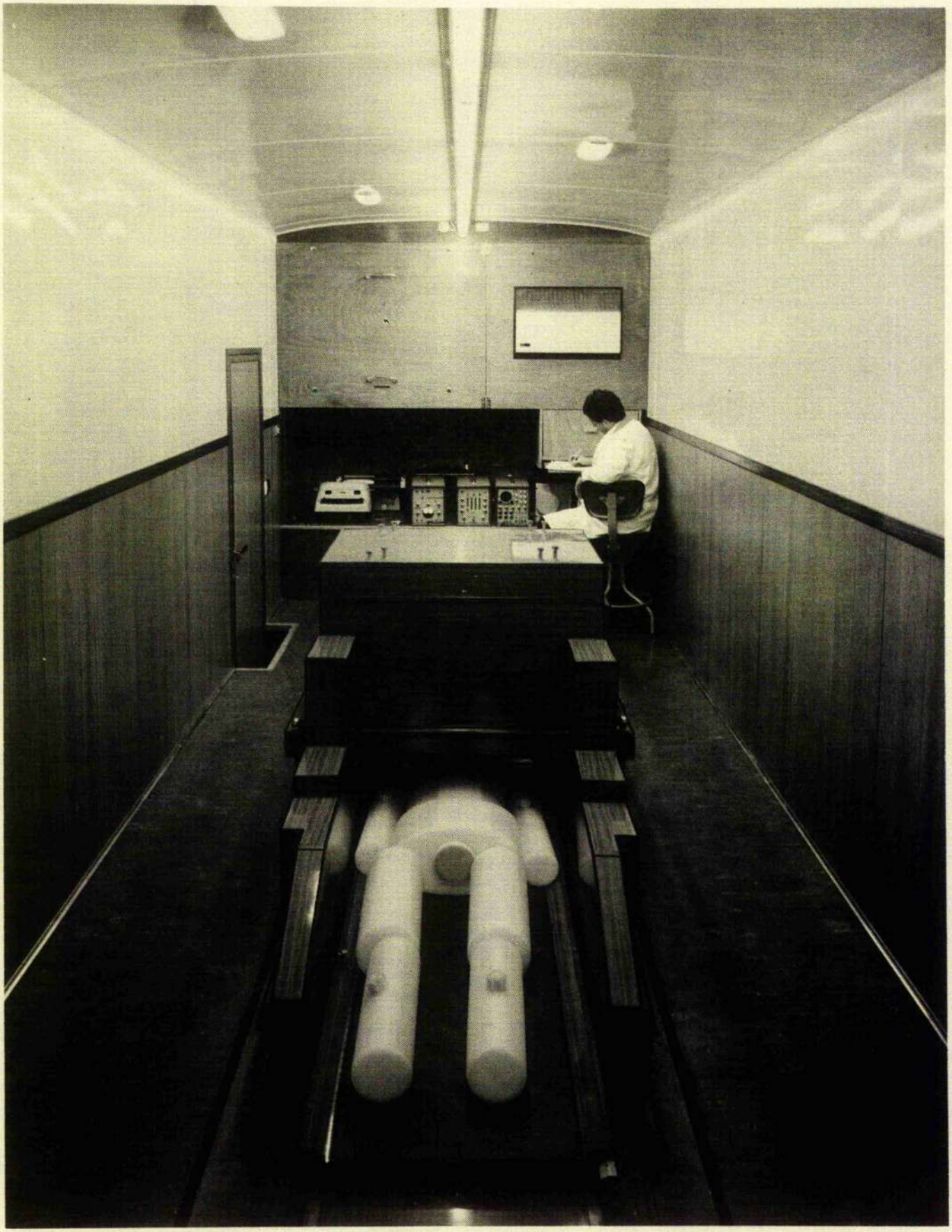
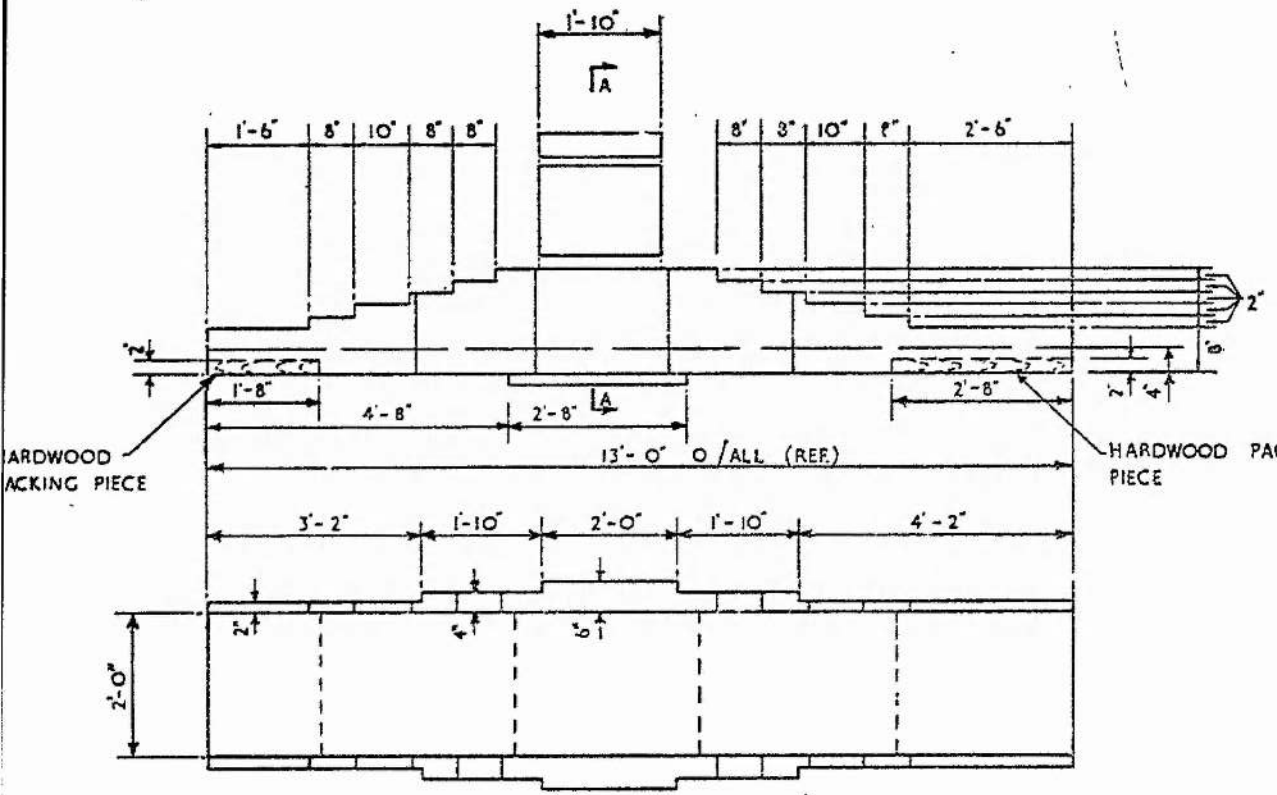


Fig. 2 MERLIN - Scanning-bed Geometry

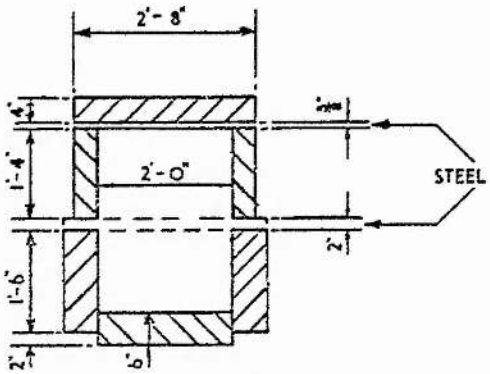
SHADOW SHIELD

Fig. 3

MATERIAL - PURE LEAD



PLAN (WITH TURRET REMOVED)



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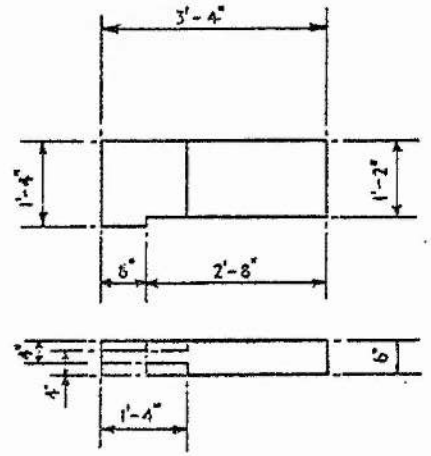
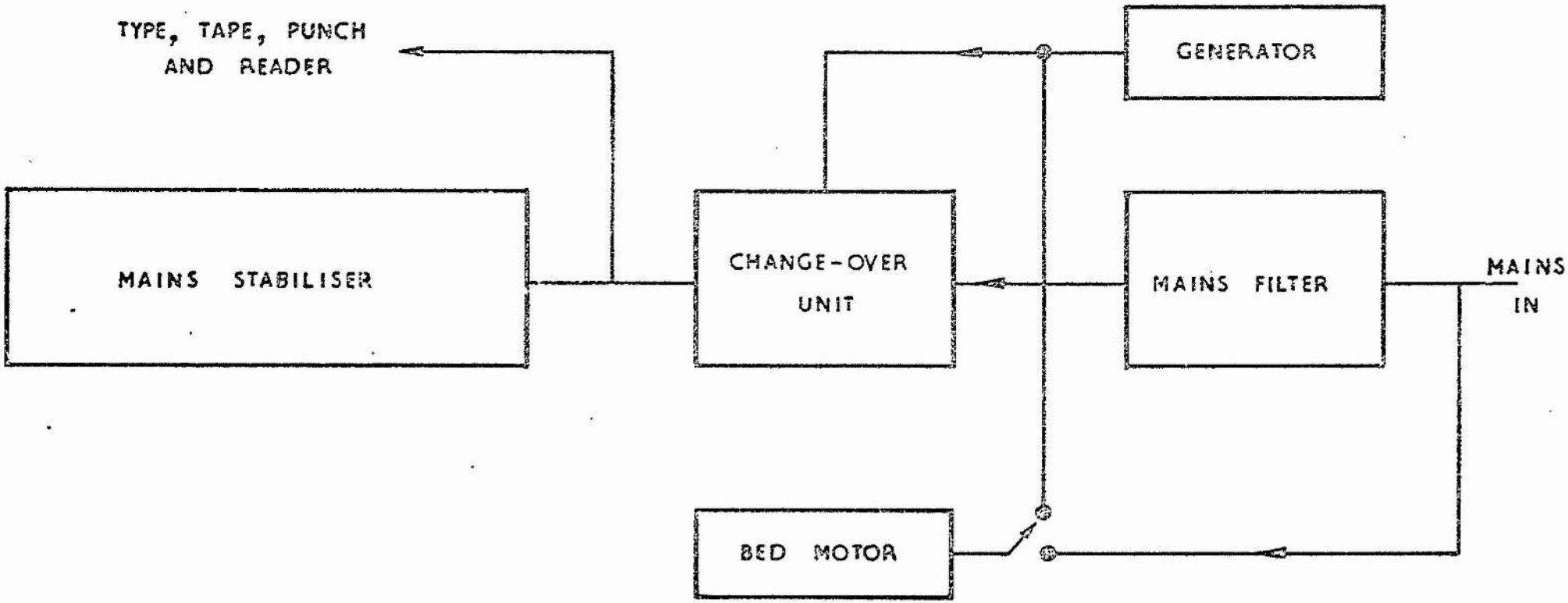


TURRET EXTENSION

1 OFF AS DRAWN
1 OFF OPP HAND (AS PER CHAIN DOT LINE)

Fig. 4 DIAGRAMMATIC PRESENTATION OF ELECTRONIC EQUIPMENT



of the motorised couch can be altered to give a minimum scanning time of 40.75 cm/min and a maximum of 4.58 cm/min. The counting is automatically started and stopped by adjustable micro-switches which are set according to the patient's height.

The scanning-bed geometry can be changed, by modification of the shield, to a tilting-chair geometry, thus accomodating patients who cannot lie flat (Boddy,1967). The tilting-chair geometry was not used for the work described in this thesis.

By using background indices (cpm per unit crystal volume), a comparison of the performance of MERLIN with that of other whole body monitors in Britain is shown in Table 1. An indication of the monitor's sensitivity for iron-59, cobalt-57, cobalt-58 and potassium-40 is presented in Table 2. A more detailed description of the monitor and of its performance has been reported by the author (King,1967). This present thesis extends this description to parameters which are of direct clinical relevance. The thesis is primarily concerned with the application of the whole body monitor in studies of vitamin B_{12} and iron metabolism, zinc uptake in prostatic carcinoma and the measurement of total body potassium.

TABLE I

Comparison of Background Indices (cpm/cc) x 10^{-2}

MONITOR LOCATION	a) Higher Energy Range.			b) Lower Energy Range.		
	ENERGY range (MeV)	Monitor Background Index (cpm/cc)x 10^{-2}	MERLIN Background Index (cpm/cc)x10^{-2}	Energy range (Mev)	Monitor Background Index (cpm/cc)x10^{-2}	MERLIN Background Index (cpm/cc)x10^{-2}
R.P.S. Sutton C.L.	1.37-1.55	4.99	1.52	0.6-0.73	18.0	2.03
A.E.E. Windscale S.	1.295-1.625	2.99	2.48	0.52-0.80	7.88	5.88
A.E.E. Winfrith S.	1.28 -1.78	3.71	3.21	0.58-0.81	6.36	4.45
A.E.E. Dounreay S.	1.27 -1.65	4.66	2.75	0.51-0.86	13.3	7.12
Univ. of Birmingham S.	1.27 -1.61	2.60	2.58	0.44-0.58	5.28	4.51
A.E.R.E. Harwell L. -multiple	1.0 -1.4	3.35	3.74	0.32-0.4	3.48	3.03
-single	do.	3.79		do.	2.94	
Addenbrookes Hosp. Cambridge S.	0.95-1.4	5.03	3.87	0.27-0.45	12.9	6.88
Radcliffe Inf. Oxford L^x	0.4 -1.5	28.0	17.0	0.17-0.44	32.9	12.1
Royal Marsden Hosp. Sutton C.L.	0.4 -1.5	76.5	17.0	0.17-0.44	59.9	12.1
General Inf. Leeds S.L.	0.1 -2.0	13.9	38.0	0.27-0.45	2.00	6.88

Shield Key: C - Chalk Room
L - Lead Room
S - Steel Room
L^x - Lead Shadow-Shield

TABLE 2

Performance data for scan on patient [+] or phantom [*]

ISOTOPE	ENERGY RANGE MeV	cpm / μCi	BACKGROUND cpm
1. Detector in high position			
Fe - 59 [+]	0.4 - 1.38	14,800	1055
	1.0 - 1.38	6,900	238
Co - 57 [+]	0.03 - 0.17		
Co - 58 [+]	0.3 - 1.0	13,000	1104
	0.74 - 0.89	7,000	153
2. Detector in low position			
K - 40 [*]	1.36 - 1.56	0.96	117
Fe - 59 [+]	0.4 - 1.38	27,900	1150
	1.0 - 1.38	13,300	260

[*] cpm / gK in phantom.

CHAPTER II

ASSESSMENT OF GEOMETRICAL VARIATIONS IN COUNTING-RATES

INTRODUCTION

Whole body counting-rates vary when administered radio-isotopes are redistributed in the body. A proper design of of whole body monitor will help to minimise the problems of whole body radioactivity measurements. A detector-to-body geometry should be chosen aiming for a response of the counter to photons emitted from the body as independent of body build and the distribution of the radioactivity within the body as possible.

Several methods of assessing the magnitude of these geometrical variations have been studied, beginning with point sources in simple water phantoms (Boddy, 1966) and progressing to measurements with a life-like phantom man with a human skeleton and compartments simulating various organs of the body (Boddy et al., 1969 A). Studies with human subjects, as the most realistic assessment, were obtained by the routine use of the whole body monitor.

A preliminary study showing the redistribution effects of iron-59, with an analysis of the resulting errors, in the phantom

man and in a patient have been reported by the author (King, 1967).

MATERIALS AND METHODS

The longitudinal and lateral variations were studied using an ampoule containing sodium-22 in a simple water phantom, placed at regular intervals along the longitudinal and lateral axes of the monitor at the height of the patient mid-line. Energy bands corresponding to the 0.51 MeV and 1.28 MeV gamma rays were used.

The variation of the counting-rate with depth in the body was investigated using a 20 cm deep water phantom, placed directly beneath the detector, and a small planar cobalt-60 source. Results were obtained with the detector in both the high and low positions. Energy bands appropriate to the photopeak and also a wide band (Warner and Oliver, 1966) were chosen.

In the measurements with an Alderson REMAB phantom,* each organ was filled in turn with a solution of iodine-131 and, subsequently, with iron-59. The remaining organs including the whole body were filled with water. Scans were then made, with the phantom in both the supine and the prone position, starting and ending at the crystal axis and at 46cm from this axis. A

* Kindly loaned by Berkeley Nuclear Laboratories, Central Electricity Generating Board, Berkely, Gloucestershire.

background measurement was made on the phantom before each organ was filled and the results corrected for any residual radioactivity. The relative amounts of radioactive solution dispensed into each organ was checked using a sodium iodide well crystal.

An extensive investigation of the redistribution effects 'in vivo' using nine different radioisotopes has been made. The patients were scanned several times up to 8 hours post-administration. The changes in the counting-rate after an oral dose of iron-59, cobalt-57, cobalt-58 and calcium-47, an intravenous dose of technicium-99m, cobalt-57, zinc-65, potassium-42, potassium-43 and sodium-24 and an intramuscular dose of cobalt-57 and cobalt-58 have been studied.

RESULTS

By graphical integration of the results obtained with the sodium-22 source placed along the longitudinal axis, a simulated scan due to the source travelling from a given point to the crystal axis is derived. Interpretation of this allows the prediction of the counting-rate that would be obtained if all the activity was localised in one organ. It was found that if the total activity was in the thyroid gland the result differed by less than 1% from the total integrated whole body count. If, however, all the activity were in a central organ, such as the liver or stomach, the integrated count is about 9% higher than

the whole body count. By increasing the length of the scan the whole body count will be increased and the central organ count will not change. However other factors also require consideration.

The investigation of the lateral variation showed a reduction in counting-rate at 7.6 cm off the axis of 97% (crystal high) and 93% (crystal low) of the axial counting-rate.

Reciprocal results of the depth variation experiment were summed to simulate supine and prone patient monitoring. The counting-rate from sources near the surface is about 1.2 to 1.5 times that at the mid-line.

The Alderson phantom results are summarised in Table 1. The ratios given are the counting-rates per unit radioactivity normalised to the value obtained when the radioactive solution was distributed uniformly throughout the whole phantom. Maximum ratios of about 1.25 were obtained for the thyroid and kidneys, both being organs close to the body surface. The results were altered little by differences in the detector position. Extention of the scanning length increased the thyroid to total body ratio for both isotopes and in practice would increase the redistribution effects. The standard deviations for the wide energy band ratios with iron-59 are consistantly lower than for the narrow band. This effect has also been found in clinical studies.

TABLE 1

COUNTING-RATE PER UNIT ACTIVITY RELATIVE TO THE TOTAL BODY USING THE ALDERSON PHANTOM

Isotope	Iodine-131						Iron-59									
Scan Condition	±46 cm		MEAN	Mid-line Scan		MEAN	±46 cm				MEAN	Mid-line Scan				MEAN
Energy Range	0.30-0.42 MeV			0.30-0.42 MeV			W	N	W	N		W	N	W	N	
Detector / Organ	High	Low		High	Low		High		Low			High		Low		
Total body	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Bladder	1.02	1.10	1.06	1.15	1.09	1.12	1.15	1.10	1.20	1.16	1.15	1.10	1.10	1.18	1.12	1.13
Thyroid	1.22	1.26	1.24	1.14	1.10	1.12	1.13	1.19	1.19	1.25	1.19	1.06	1.12	1.04	1.11	1.09
Pancreas	0.96	1.07	1.02	0.98	0.93	0.96	1.09	1.04	1.05	1.00	1.05	1.05	1.03	1.07	1.01	1.04
Spleen	0.91	0.89	0.90	1.01	1.02	1.02	1.12	1.09	1.01	0.97	1.05	1.03	1.05	0.98	0.92	1.00
Liver	0.95	1.07	1.01	1.00	1.02	1.01	1.09	1.04	1.08	1.03	1.06	1.06	1.03	1.08	1.02	1.05
Kidneys	1.17	1.22	1.20	1.16	1.18	1.17	1.17	1.15	1.15	1.14	1.15	1.16	1.16	1.12	1.09	1.13
Stomach	1.04	1.04	1.04	1.04	0.90	0.97	1.12	1.08	1.10	1.04	1.09	1.05	1.02	1.05	0.98	1.03
Mean	1.03	1.08	-	1.06	1.03	-	1.11	1.09	1.10	1.07	-	1.06	1.06	1.07	1.03	-
σ (% of Mean)	±10.54	±10.88	-	±7.25	±8.89	-	±4.68	±5.66	±6.98	±9.21	-	±4.53	±5.34	±5.99	±6.80	-

σ = Total standard deviation

W = Wide Energy Band (0.40-1.38 MeV)

N = Narrow Energy Band (1.00-1.38 MeV)

The 'in vivo' results following the oral administration of radioactive iron in 5 patients are given in Table 2. The detector was used in the high and low positions and the scans were made beginning and ending at the crystal axis and at 46 cm from this axis. The total standard deviation for the ± 46 cm scan is greater than the crystal axis scans because of the increased error due to the counting statistics. The standard deviation is 2.2% for the extended scan when the counting statistics are omitted. For subjects A, B and C, the average standard deviations were 5.6% (range 2.2 - 8.1) for supine measurements only and 5.9% (range 3.7 - 8.8) for prone measurements only. Vitamin B_{12} labelled with cobalt-57 or cobalt-58 was given orally and the subjects monitored frequently on the day of administration. The results are given in Table 3, the 40 minute count being taken as the 100% value. The standard deviations are small and the results show that for the few patients who cannot be monitored in the prone position (less than 2%) the standard deviations are ± 6.8% for cobalt-58 and ± 5.3% for cobalt-57. Calcium-47 was administered orally to 4 subjects and the initial variations in the counting-rates studied. The results, given in Table 4, show that the counting-rate at about 2 hours post-administration generally increases by about 10% and from 2 hours onwards it remains remarkably constant up to 7 hours post-administration. Taking a mean of the counting-rates

TABLE 2

VARIATIONS IN COUNTING-RATES

FOLLOWING ADMINISTRATION OF ORAL IRON

± 46 cm			Mid-Line Scan											
Detector High			Detector Low			Detector High								
						Patient A			Patient B			Patient C		
Time Hrs	W	N	Time Hrs	W	N	Time Hrs	W	N	Time Hrs	W	N	Time Hrs	W	N
0	100.0%	100.0%	0	100.0%	100.0%	0	100.0%	100.0%	0.25	100.0%	100.0%	2.50	100.0%	100.0%
1.2	97.0	98.5	1.0	97.7	98.7	1.0	98.5	97.1	0.50	97.3	95.1	3.50	96.4	92.3
2.2	97.5	98.4	2.0	97.6	100.6	2.0	103.1	103.7	0.75	95.4	93.5	5.00	96.3	94.6
3.8	92.6	90.5	3.0	102.0	104.1	3.0	105.9	106.0	3.00	99.5	100.3	6.50	102.6	99.6
4.8	95.8	95.0	4.0	98.2	98.9	4.5	104.6	105.9	3.25	100.0	97.3	7.50	96.2	95.1
5.8	101.2	98.8	5.0	96.0	96.5	5.5	103.6	105.2	3.50	100.9	100.5	8.50	97.2	97.8
6.8	97.0	97.0	6.5	92.9	92.9	6.5	103.6	104.7				9.25	96.2	94.7
23.2	103.6	105.2										10.50	98.1	97.4
												11.50	96.4	94.8
												26.50	102.5	104.0
σ	± 3.4%	± 4.1%	σ	± 2.7%	± 3.7%	σ	± 2.4%	± 3.1%	σ	± 2.1%	± 3.0%	σ	± 2.6%	± 3.5%

W = Wide Band (0.40-1.38 MeV)
N = Narrow Band (1.00-1.38 MeV)
σ = Total Standard Deviation

TABLE 3

VARIATION IN COUNTING-RATE FOLLOWING

ORALLY ADMINISTERED COBALT-57 AND COBALT-58

LABELLED VITAMIN B_{12}

TIME HRS	^{58}Co-VITAMIN B_{12} as % of 0.7 hr result (0.3-1.0 MeV)	^{57}Co-VITAMIN B_{12} as % of 0.7 hr result (0.035-0.188 MeV)
0.1	96.2	103.7
0.7	100.0	100.0
2.0	104.7	105.8
2.8	105.8	105.9
3.4	102.1	102.9
4.1	100.1	103.3
4.7	98.3	98.3
σ	± 3.4%	± 2.7%

σ = Total standard deviation

MID-LINE SCAN, DETECTOR HIGH

TABLE 4

SERIAL WHOLE BODY COUNTING-RATES

AFTER ORAL ADMINISTRATION OF CALCIUM-47

Time Hrs.	Patient A		Time Hrs.	Patient B		Time Hrs.	Patient C		Time Hrs.	Patient D	
	W	N		W	N		W	N		W	N
0.5	100.0%	100.0%	1.0	100.0%	100.0%	0.2	100.0%	100.0%	1.0	100.0%	100.0%
2.0	109.8	112.7	2.0	107.2	112.7	1.5	104.3	109.8	2.5	101.3	103.8
4.0	108.8	113.6	4.5	106.8	113.0	4.5	104.4	110.0	5.0	97.4	101.5
5.5	107.4	113.2	6.0	106.1	111.7	6.0	103.7	107.9	6.5	96.1	99.4
6.5	106.8	112.1	7.0	105.6	111.6	7.0	104.1	109.9			
σ	$\pm$ 3.8%	$\pm$ 5.8%		$\pm$ 2.9%	$\pm$ 5.5%		$\pm$ 1.9%	$\pm$ 4.3%		$\pm$ 2.4%	$\pm$ 2.0%

W = Wide Band (0.36-1.43 MeV)

N = Narrow Band (1.14-1.43 MeV)

σ = Total standard deviation

Mid-line Scan, Detector high.

in this period as the 100% value, the absorption up to 11 days post-administration was calculated. It can be seen from Table 5 that reasonable agreement was obtained between these absorption figures and those calculated from the assay of urine and faeces.

A study of redistribution effects following intravenous administration has been made with six different radioisotopes. The detector to patient mid-line distance was 30 cm (high position) and the scans began and ended at the crystal axis. The results of repeated whole body measurements after an intravenous dose of technetium-99 m and cobalt-57 labelled vitamin B_{12} are given in Table 6. The results are very self-consistant although maximum redistribution would be expected during the monitoring periods. With technetium-99 m, the supine results are always greater than the prone, presumably due to thyroidal uptake of the isotope. This difference, however, is not large enough to cause a significant uncertainty. Satisfactory agreement was obtained between the retention of vitamin B_{12} and of technetium estimated by whole body monitoring and by radioassay of the urine collected. The results of measurements in 3 patients following the administration of zinc-65 are given in Table 7. The variation in the counting-rate for both the wide energy band and the photopeak band is very small and did not exceed $\pm$ 1.2%. The changes in the counting-rate following an intravenous dose of potassium-42, potassium-43 and sodium-24 are summarised in

TABLE 5

ABSORPTION OF ORALLY ADMINISTERED

CALCIUM-47

TIME DAYS	PATIENT A		PATIENT B		PATIENT C		PATIENT D	
	WBM	U+F	WBM	U+F	WBM	U+F	WBM	U+F
0	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
1.4	94.2	95.7	96.8	96.8	91.6	93.5	96.8	99.9
2.4	88.1	90.3	66.3	68.1	86.6	88.3	95.2	99.0
3.4	75.3	80.3	47.2	51.5	84.3	84.9	87.3	91.7
4.4	69.4	76.5	39.0	43.6	57.7	63.8	86.9	91.7
6.5	-	-	34.4	40.2	-	-	-	-
8.4	61.0	71.0	31.5	38.4	45.0	55.1	78.7	85.2
9.4	59.2	70.2	30.0	37.8	42.2	54.8	76.3	84.8
10.4	58.2	69.2	29.4	37.4	41.9	54.4	75.4	83.6.
11.4	57.2	68.4	28.9	36.9	40.8	53.9	74.6	83.6

U+F = % ABSORPTION BASED ON ASSAY OF URINE AND FAECES.

TABLE 6

RESULTS OF REPEATED WHOLE BODY MEASUREMENTS FOLLOWING INTRAVENOUS ADMINISTRATION OF TECHNETIUM-99m AND COBALT-57 LABELLED VITAMIN B_{12}

TIME AFTER ADMINISTRATION	TECHNETIUM-99m 0.03-0.17 MeV	COBALT-57 0.035-0.188 MeV
0-2 HOURS	100.0%	100.0%
	100.5%	97.7%
	$\sigma = \pm 0.4\%$	$\sigma = \pm 1.6\%$
	URINE PASSED	
2-4 HOURS	92.7%	82.9%
	91.4%	82.1%
	$\sigma = \pm 0.9\%$	82.4%
		$\sigma = \pm 0.6\%$
	URINE PASSED	
4-6 HOURS	79.1%	81.4%
	78.5%	79.5%
	$\sigma = \pm 0.4\%$	81.0%
		$\sigma = \pm 1.4\%$

σ = Total standard deviation.

TABLE 7

SERIAL WHOLE BODY COUNTING-RATES AS PERCENTAGE OF INITIAL RESULT FOLLOWING INTRAVENOUS ZINC-65

TIME (HOURS)	PATIENT A		PATIENT B		PATIENT C	
	W	N	W	N	W	N
3.5	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
5.5	100.8	101.8	101.2	101.8	99.0	100.1
8.5	99.3	99.5	100.6	100.6	100.1	100.0
σ =	± 0.8%	± 1.2%	± 0.6%	± 0.9%	± 0.6%	± 0.1%

W = Wide band (0.44-1.28 MeV)

N = Narrow band (0.91-1.28 MeV)

σ = Total standard deviation

Table 8. Because of the nature of the clinical study for which the isotopes were administered, it was only possible to monitor the patients for a maximum of 3 hours post-injection. No excessively large redistribution effects were observed in this period.

Vitamin B_{12} labelled with cobalt-57 or with cobalt-58 was given intramuscularly and the subjects monitored frequently on the day of administration. The results are given in Table 9. The standard deviations are small and again the retention calculated from the whole body measurements and from radioassay of the urine gave good agreement.

The maximum spread in counting-rates expressed as a ratio was calculated for each patient and separate isotope. Following intravenous administration the mean ratios ranged from 1.01 to 1.03. The mean ratios for intramuscular cobalt-57 and cobalt-58 were 1.05 and 1.07 respectively. Orally administered isotopes gave the highest ratios, the mean values ranging from 1.07 for iron-59 to 1.10 for cobalt-58. The result for iron-59 was calculated using the wide energy band data from the mid-line scan with the detector in the high position. The Alderson phantom results indicated these to be the optimum monitoring conditions for iron-59 when redistribution of the isotope is expected and are the conditions used for routine patient monitoring.

TABLE 8

VARIATIONS IN WHOLE-BODY MEASUREMENTS FOLLOWING INTRAVENOUS POTASSIUM-42, POTASSIUM-43 AND SODIUM-24

Time (Hours)	Potassium-42		Time (Hours)	Potassium-43	Time (Hours)	Potassium-43	Time (Hours)	Sodium-24	
	W	N		Patient A		Patient B		W	N
0.25	100.0%	100.0%	0.25	100.0%	0.75	100.0%	0.3	100.0%	100.0%
1.25	97.9	99.2	1.25	100.9	2.0	98.4	1.0	96.1	94.2
2.25	97.4	99.1	2.25	99.8	3.0	100.8			
σ=	$\pm$ 1.4%	$\pm$ 0.5%	σ=	$\pm$ 0.6%	σ=	$\pm$ 1.2%	σ	$\pm$ 2.8%	$\pm$ 4.1%

Energy Bands:

Potassium-42, W = (0.77-1.27 MeV)
N = (1.42-1.60 MeV)

Potassium-43, (0.26-0.73 MeV)

Sodium-24, W = (1.15-1.57 MeV)
N = (2.48-2.95 MeV)

σ = Standard Deviation

TABLE 9

CHANGES IN THE COUNTING-RATE ON THE DAY OF INTRAMUSCULAR INJECTION OF COBALT-57 HYDROXOCOBALAMIN AND COBALT-58 CYANOCOBALAMIN

TIME AFTER INJECTION (HRS)	^{57}Co-OHB_{12} (0.035-0.188 MeV)	^{58}Co-CNB_{12} (0.3-1.0 MeV)
0	100.0%	100.0%
0.75	100.2	99.7
1.5	99.8	101.1
2.0	104.6	104.5
σ =	± 2.3%	± 2.2%
	URINE PASSED	
3.5	66.4%	42.4%
5.0	68.1	45.5
5.75	69.5	44.8
6.75	67.9	46.2
σ =	± 1.3%	± 1.7%

MID-LINE SCAN, DETECTOR HIGH

σ = Total standard deviation

DISCUSSION

Measurements made with point sources showed that changes in the depth of an isotope within the body are the principle source of counting-rate variations. The study of longitudinal variation with a point source at the patient mid-line showed that extending the length of the scan beyond the extremities of the subject would make an insignificant improvement in the counting-rate variations. Because depth variation is so much more significant, an extended scan in the case of a superficial organ such as the thyroid would tend to increase rather than reduce the variations. The depth variation suggest that for small surface organs such as the thyriod and kidneys, the organ to total body ratio should not exceed 1.3 if all the isotope were either in the organ or in the total body. This ratio would be less for the bladder which is also a surface organ but of greater depth.

The predictions from the point source experiments were confirmed by the results with the Alderson phantom. Surface organs gave higher organ to total body ratios, the maximum ratio being 1.26 for an extended scan of the phantom with the detector in the low position and iodine-131 in the thyroid gland. The extended scans always resulted in greater thyroid to total body ratios than the mid-line scans. The standard deviations for the organ to total body ratios with iron-59, calculated using the wide energy band, were smaller than those using the photopeak band.

Using the thyroid, bladder, liver and total body of the Alderson phantom, Naversten (1964) found similar variations (1.00 - 1.21) for iodine-131 in a two-crystal scanning bed geometry where the detector faces were 70 cm apart. Dudley and Ben Haim (1968) using a crystal geometry similar to that of MERLIN, except the detector moves relative to the patient, studied the redistribution effects with a polythene phantom of cylinders filled with iodine-131. Variations of 1.00-1.14 were reported by the authors, these probably being less than those by Naversten and ourselves since the cylinders of the phantom did not have simulated organs of varying depth.

The results from both the point source and the Alderson phantom study give a pessimistic estimate of the redistribution errors. In practice, an isotope is rarely transferred completely from the total body to one single organ or vice versa. The limitations in the interpreting of these results are the assumptions required to make the most realistic estimate of the variations in counting-rates that would be obtained in practice.

The 'in vivo' study appears to be among the most extensive reported with respect to the range of isotopes and, with labelled vitamin B_{12}, the routes of administration. The results are in reasonable agreement with the conclusions from both the point source and the phantom studies, although, as suggested, the redistribution errors in practice may be lower than those

predicted. Oral administration of the radioisotopes studied resulted in the largest redistribution errors and the spread in the results gave the highest maximum ratios. The transfer of labelled vitamin B_{12} from the stomach to the liver would be expected to produce larger variations than after intravenous administration where very rapid clearance to the liver occurs with a half life of a few minutes. The ratios after intramuscular administration of labelled vitamin B_{12} were lower than those for orally administered isotopes but higher than those after intravenous administration. This presumably results from the initial rapid clearance to the liver of some of the isotope from the site of injection and the remainder being a more dispersed source at a depth of several inches below the body surface. An extended scan with iron-59 did not significantly reduce the variation in counting-rate due to isotope redistribution and the total standard deviation was higher than for the mid-line scans. Even for cases where an extended scan would improve the reproducibility it is doubtful whether this justifies the resulting decrease in sensitivity and an increase in monitoring time. With no isotope were the redistribution effects prohibitively large using only the supine or the prone scanning position, the results generally being clinically acceptable.

SUMMARY

Several methods of assessing the magnitude of geometrical variations in counting-rates have been brought together in this study. Point sources in simple water phantoms, a life-like phantom containing simulated organs and human subjects were studied and the relative importance of the sources of variation and the value of the methods assessed. The results from all methods were in reasonable agreement and showed that realistic estimates of the variation in the counting-rate can be obtained by 'in vitro' studies.

CHAPTER III

EFFECT OF RENAL AND HEPATIC DISEASE ON VITAMIN - B_{12} METABOLISM

INTRODUCTION

Adams and Boddy (1968) showed that by 5 to 10 days after an intravenously administered tracer dose of 0.1 µg of cyanocobalamin, the rate of loss from the body could be adequately described by a single exponential term. This and other evidence suggest that subsequent to about a week after injection the cobalamin had effectively equilibrated with body stores (Will et al., 1969). The significance of this finding relates to a method for measuring the effect of factors such as disease, drugs or nutrients on cobalamin metabolism, on a relatively short-term basis. This had not previously been regarded as a practical proposition. The authors recommend measuring the whole-body retention from 7 days until 30 days after injection if a standard error of about 20 to 30% is acceptable or until approximately 60 days if a standard error of about 10% is required.

Using this procedure, the cobalamin loss has been investigated in patients with renal disease and hepatic disease. Since most of the vitamin B_{12} in the body is stored in the liver and is excreted via the urine and bile, diseases of the liver and kidneys might therefore be expected to affect the cobalamin metabolism.

PATIENTS AND METHODS

Measurements have been made on 8 patients with renal disease and 6 patients with hepatic disease. The biochemical and clinical data for patients with renal disease are given in Table 1 and for patients with hepatic disease in Table 2. Cases 1 to 6 came under observation with symptoms attributable to hypertension and case 7 presented in uraemic coma, being later diagnosed as having renal tubular acidosis due to chronic pyelonephritis. Case 8 presented with signs and symptoms of diabetes and diabetic nephropathy was diagnosed after a renal biopsy. Patient 4, who was addicted to phenacetin, had undergone right nephrectomy for hydronephrosis and renal abscesses 5 years previously. A renal biopsy on case 5 showed chronic glomerulonephritis and in case 3 there was evidence of chronic pyelonephritis. The patients with liver disease had presented in a variety of ways and primary biliary cirrhosis was diagnosed in cases 9 to 13 on the basis of the clinical picture, biochemical data, microscopy of the liver tissue obtained by percutaneous biopsy or at laparotomy, and a positive mitochondrial-antibody test. Case 14 presented with portal hypertension and was diagnosed as having nodular cirrhosis. Case 9 had previously undergone cholecystectomy and case 10 had radiological pulmonary fibrosis and had recurrent uninary-tract infections. Six years previously, case 14 had an emergency porto-caval shunt performed for bleeding

TABLE 1

CLINICAL AND BIOCHEMICAL DATA IN PATIENTS WITH RENAL DISEASE

Case No.	Age	Sex	Hb (g per 100 ml)	Serum-Vitamin-B_{12} (pg per ml)	Serum Folate (ng per ml)	Blood-Urea (mg per 100 ml)	Urea Clearance (%)	Blood-Pressure (mm Hg)	Albumin-uria	Renal Radiology	Drug Therapy
1	64	M	11.0	360	3.0	130	14.0	270/120	+++	L 11.0 cms, R 11.5 cms; diffuse loss of substance	NIL
2	85	F	8.7	430	32.0	50	12.6	200/100	+	L 9.5 cms, R 9.5 cms; diffuse loss of substance	NIL
3	57	F	10.4	182	12.4	70	24.0	200/105	-	L 11.0 cms, R 11.0 cms; focal loss of substance	Methyldopa, Warfarin, Bendrofluazide
4	59	F	10.9	630	2.6	70	8.1	230/140	++	Probable focal loss of substance	Methyldopa, Bendrofluazide
5	38	M	12.6	> 1000	-	80	28.0	150/110	++	L 14.0 cms R 14.5 cms	Guanethidine Bendrofluazide
6	49	M	16.2	776	8.4	78	40.0	200/100	+	L 11.0 cms, R 12.5 cms	Guanethidine, Potassium Chloride, Bendrofluazide
7	54	F	11.0	410	6.0	40	20.0	120/80	-	L 12.0 cms, R 10.0 cms; focal loss of substance	Ampicillin, Sodium Bicarbonate, Potassium Chloride, Imipramine
8	51	F	11.7	-	-	46	23.2	180/80	+++	L 14.5 cms, R 14.0 cms.	Soluble and Zinc Protamine Insulin, Lasix, Cycloserine, Aldactone A.

TABLE 2

CLINICAL AND BIOCHEMICAL DATA IN PATIENTS WITH HEPATIC DISEASE

Case No.	Age	Sex	Hb (g. per 100 ml.)	Serum-Vitamin B_{12} (pg. per ml.)	Serum-Folate (ng per ml.)	Serum-Bilirubin (mg. per 100 ml.)	Bromsulphophthalein Retention %	Serum-Albumin (g. per 100 ml.)	Serum-Globulin (g. per 100 ml.)	Serum Transaminases (Dade Units)		Serum-Alkaline Phosphatase (King-Armstrong Units)	Thymol Turbidity (Units)	Zinc-Sulphate Turbidity (Units)	Serum-Cholesterol (mg. per 100 ml.)	Drugs
										Glutamic/ Oxaloacetic	Glutamic/ Pyruvic					
9	49	F	11.5	840	6.2	10.8	54	3.2	2.7	259	124	42	4.4	5.2	410	Azathioprine, Frusemide, spironolactone, Cholestyramine
10	69	F	12.4	760	5.2	0.6	14	3.6	3.6	45	30	39	12.0	16.0	440	Azathioprine, Prednisone, Ampicillin
11	55	F	13.9	400	14.2	1.9	38	2.6	2.6	53	38	27	27.6	22.4	221	Azathioprine, Prednisone, Neomycin
12	59	F	8.7	290	3.2	0.7	17	4.0	4.1	95	81	49	6.0	20.8	372	Prednisone, Vitamin K
13	53	F	11.6	360	5.2	0.4	3	3.4	3.9	90	44	22	6.4	26.4	261	Azathioprine, Prednisone
14	59	F	11.5	888	-	1.3	-	2.5	2.9	62	31	16	4.2	12.6	-	Neomycin

oesophageal varicies.

Baseline biochemical and haematological investigations were carried out on all the patients before the intravenous administration of 0.1 μg of cyanocobalamin in 3.0 ml of water. In cases 1 to 4, 7 and 9 to 13 the vitamin B_{12} was labelled with 0.5 μCi of cobalt-58 and in the remainder with 1.0 μCi of cobalt-57. The initial whole body measurement was made shortly after administration and was taken as the 100% value. The patients were monitored again one week later and subquently at regular intervals until adequate data had been obtained. The excretion rates from day 7 onwards were calculated by the method of least squares. A direct link to the 'UNIVAC 1108' computer of the National Engineering Laboratories is installed at the Centre and was used to aid the computations.

RESULTS

The experimental data and the calculated excretion-rates with their standard deviations for each patient are presented in Table 3.

DISCUSSION

The long-term excretion-rate of cyanocobalamin, hydroxocobalamin or coenzyme B_{12} in patients with vitamin B_{12} deficiency is 0.1 - 0.2% per day (Boddy and Adams, 1968). Normal subjects

TABLE 3

EXCRETION-RATES OF 0.1 μg VITAMIN B_{12} IN PATIENTS WITH RENAL AND HEPATIC DISEASE

CASE No.	PERIOD OF STUDY (DAYS)	No. OF OBSERVATIONS	EXCRETION-RATE (% PER DAY)	STANDARD DEVIATION ON ESTIMATE
RENAL DISEASE				
1	98	9	0.175	± 0.020
2	63	5	0.267‡	± 0.039
3	67	11	0.178	± 0.042
4	74	8	0.510‡	± 0.038
5	50	6	0.356‡	± 0.120
6	80	8	0.276‡	± 0.037
7	99	9	0.328‡	± 0.028
8	93	10	0.333‡	± 0.035
HEPATIC DISEASE				
9	66	8	0.126	± 0.037
10	39	5	0.291‡	± 0.085
11	36	4	0.381‡	± 0.079
12	39	5	0.302‡	± 0.045
13	36	4	0.185	± 0.018
14	98	5	0.158	± 0.082

‡ ABNORMALLY HIGH VALUES.

given 0.1 μg of cyanocobalamin had a loss-rate of 0.15 - 0.20% per day (Adams and Boddy, 1968). Thus six out of the eight patients studied with renal disease and three out of the six with hepatic disease had an abnormally high excretion-rate. Although these data do not prove that the diseases are the cause of the abnormal loss of cobalamin, it is at least suggestive evidence that this is the case. It is unlikely that this would be caused by drug therapy, although the possibility can only be definitely exclused in case 2. If the diseases are the cause of the high excretion-rates, it is not clear why in some cases the daily loss was normal when clinically and biochemically the disease process was more severe than in patients with an abnormal loss.

The human requirement for cobalamin can be regarded as the amount which must be absorbed in order to maintain body stores. With a loss of 0.1 - 0.2% per day, the amount required to maintain balance will be 0.1 to 0.2% of body stores per day. If the total body stores are between 1000 and 5000 μg, then the need will range from 1 to 10 μg daily. This is in agreement with an estimated dietary intake of vitamin B_{12} of between 2 and 9 μg per day by Heyssel et al. (1966). When the loss rises to 0.51% per day, as in case 4, the daily requirement for maintenance of stores will range from 5.1 to 25.5 μg. The implication of such a value in relation to the cobalamin nutrition of patients with renal and hepatic disease is obvious and requires further investigation.

SUMMARY

Cobalamin excretion-rates were studied in patients with renal and hepatic disease. The daily loss was abnormally high in six out of eight patients with renal disease and three out of six with hepatic disease. The cause of this abnormality was not identified. The results imply that some patients with renal and hepatic disease have a greater requirement for cobalamin than both normals and patients with vitamin B_{12} deficiency if balance is to be maintained.

CHAPTER IV

RETENTION OF CYANOCOBALALAMIN, HYDROXOCOBALAMIN AND COENZYME B_{12} AFTER PARENTERAL ADMINISTRATION IN EVALUATING THE TREATMENT OF VITAMIN B_{12} DEFICIENCY

INTRODUCTION

A frequent method for predicting the results of vitamin B_{12} therapy in patients with pernicious anaemia is by monitoring the urine passed for about 3 days post-injection. Studies on patients with vitamin B_{12} deficiency treated with single 5000 µg doses of cobalamins (Boddy and Adams, 1968) showed that by using this method the loss would be underestimated, since between day 3 and the point at which a steady-state is attained (after a month or more post-injection) considerable B_{12} loss occurs in the urine and faeces. In terms of daily loss it is small and unlikely to be detectable by the monitoring of daily excreta collections, but is apparent on whole body measurements. This period of relatively rapid loss has not previously been studied with regard to its therapeutic implications. For this reason it was decided to investigate the retention of cyanocobalamin, hydroxocobalamin and coenzyme B_{12} after parenteral administration and to determine treatment schedules for patients with vitamin B_{12} deficiency.

'In vivo', both cyancoobalamin and hydroxocobalamin are

believed to be converted to coenzyme B_{12} (α-[5 : 6 - dimethylbenziminazolyl] 5' - deoxyadenosyl cobamide) (Rosenblum et al. 1960, Pawelkiewicz et al. 1964, Uchino et al. 1965). In a previous study (Boddy and Adams,1968) the results from one patient suggested that for the month after injection of coenzyme B_{12} the pattern of loss differed from that of cyanocobalamin and hydroxocobalamin. Because of this difference and because coenzyme B_{12} may be of therapeutic importance, this analogue was included in the study.

MATERIALS AND METHOD

The 24 volunteers studies were either normal or had irrelevant diseases. All had a normal serum vitamin B_{12} level and normal peripheral blood values and no patient had renal or hepatic disease. The patients were divided into two groups, the first receiving ^{58}Co - cyanocobalamin and ^{57}Co - hydroxocobalamin and the second ^{58}Co - cyanocobalamin and ^{57}Co - coenzyme B_{12}. The mass of each dose was 1000 μg labelled with 0.5 μCi of either cobalt-58 or cobalt-57. Administration was by injection into the buttock and in each group the procedure was the same. The cyanocobalamin was given on day 0 and the retention was measured immediately after injection to give a 100% value, and again at 3 and 28 days after administration, corrections being made for natural body radioactivity, background and radioactive decay.

A week after the injection of cyanocobalamin, hydroxocobalamin or coenzyme B_{12} was given into the opposite buttock and the retention measured immediately and at 3 and 28 days after injection. In evaluating the results, corrections were made for the contribution in the Compton region of the cobalt-58 gamma ray spectrum to the photopeak of the cobalt-57 spectrum, this being calculated for each subject. The amount of isotope remaining at the site of injection was determined by surface counting, allowances being made for the background radiation.

The doses of ^{58}Co - cyanocobalamin and ^{57}Co - hydroxocobalamin were prepared with their respective commercially available stable cobalamins. The stable and ^{57}Co - coenzyme B_{12} solutions were prepared by the methods described by Johnson et al. (1963), and the doses for injection were processed in dim red light and then shielded from direct light by wrapping the ampoules and syringes in heavy foil. The preparations were sterilised by millipore filtration.

RESULTS

By 3 days after administration, the whole body retention of coenzyme B_{12} was 40.9% and of cyanocobalamin was 15.1%. A Student's t test showed their difference to be statistically significant with an uncertainty of less than 0.1% ($P < 0.001$). The subsequent loss of coenzyme B_{12}, however, was more rapid

than that of cyanocobalamin, so that by 28 days after administration the mean retention of coenzyme B_{12} was 18.5% compared with 13.6% for cyanocobalamin ($0.01 > P > 0.001$). The results for the individualpatients are shown in Table 1.

The retention of hydroxocobalamin on the 3rd day, 42.1%, was more than three times that of cyanocobalamin, 13.7% ($P < 0.001$). By the 28th day, the retention of hydroxocobalamin was still more than three times the retention of cyanocobalamin, the values being 32.5% and 10.5% respectively ($P < 0.001$). Therefore, unlike that of coenzyme B_{12}, the fractional losses of hydroxocobalamin and cyanocobalamin between day 3 and 28 were practically identical. The results for the individual patients are shown in Table 2.

There was no difference between the mean values for the retention of hydroxocobalamin and coenzyme B_{12} by day 3, but by day 28 the amount of hydroxocobalamin retained was significantly greater ($P < 0.01$).

The variation in the counting-rate on the day of administration was small, the typical redistribution effects being shown in Table 9, Chapter II. The advantage of small variations in the initial counting-rate is that uncertainty in the 100% value and in the fraction of cobalt-58 gamma rays detected in the cobalt-57 energy band is reduced. Boddy et al. (1969B) estimated that the uncertainty in this fraction and in the

TABLE 1

WHOLE BODY RETENTION OF ^{58}Co-CYANOCOBALAMIN AND ^{57}Co-COENZYME B_{12}

CASE No.	% RETENTION OF ^{58}Co-CNB$_{12}$		% RETENTION OF ^{57}Co-CoEB$_{12}$	
	DAY 3	DAY 28	DAY 3	DAY 28
1	16.2	15.0	37.0	22.0
2	22.2	-	53.7	-
3	14.0	-	44.2	-
4	13.8	11.6	55.6	16.8
5	10.5	-	29.8	-
6	15.6	14.0	36.6	17.2
7	19.3	-	53.4	-
8	12.3	10.8	40.2	17.1
9	19.4	16.7	42.0	19.3
10	12.7	-	33.5	-
11	9.6	-	23.8	-
MEAN (%)	15.1	13.6	40.9	18.5
MEAN (μg B_{12})	151	136	409	185

TABLE 2

WHOLE BODY AND SITE RETENTION OF ^{58}Co-CYANOCOBALAMIN AND ^{57}Co-HYDROXOCOBALAMIN

CASE No.	% RETENTION OF ^{58}Co-CNB$_{12}$			% RETENTION OF ^{57}Co-OHB$_{12}$		
	WHOLE BODY		SITE	WHOLE BODY		SITE
	DAY 3	DAY 28	DAY 3	DAY 3	DAY 28	DAY 3
1	12.4	8.5	0.4	25.0	18.9	3.4
2	12.2	10.2	1.0	29.9	26.0	1.7
3	16.2	11.4	-	62.1	46.0	-
4	11.1 *	8.4	-	50.1	34.2	0.3
5	12.6 *	10.3	-	-	35.5	-
6	12.4	9.9	0.4	28.5	20.9	2.4
7	11.5	8.3	0.6	50.0	37.5	4.6
8	12.5	9.9	0.6	49.9	38.5	1.3
9	17.2	12.8	0.7	42.4	31.3	3.1
10	16.8	15.2	0.6	44.3	35.7	1.2
11	14.7	-	0.4	39.0	-	1.4
12	13.0	-	0.5	-	-	-
13	15.3	-	0.3	-	-	-
MEAN (%)	13.7	10.5	0.6	42.1	32.5	2.2
MEAN (μg B_{12})	137	105	6	421	325	22

* Day 4

cobalt-58 counting-rate results in an uncertainty of about 4% in the counting-rate due to cobalt-57.

DISCUSSION

The 3 and 28 day retention patterns of cyanocobalamin, hydroxocobalamin and coenzyme B_{12} after parenteral administration are quite different and have not previously been recorded. The results of the measurements at the site of injection show that the difference between the retention of cyanocobalamin and hydroxocobalamin by day 3 cannot be explained by unequal rates of absorption from the site. There is evidence that the bulk of the body stores of vitamin B_{12} are in the coenzyme form (Toohey and Barker 1961, Stahlberg et al. 1967). It has also been shown that cyanocobalamin and hydroxocobalamin are converted 'in vivo' to coenzyme B_{12} (Rosenblum et al. 1960, Pawelkiewicz et al. 1964; Uchino et al.1965) and that by about a month after administration all three forms have an apparently identical excretion-rate (Boddy and Adams, 1968). Since dietary vitamin B_{12} is in the form of cyanocobalamin or hydroxoxobalamin which, after absorption, is presumably transferred to the body stores for conversion to coenzyme B_{12}, it might be expected that the metabolism of these two vitamin B_{12} analogues would be qualitatively similar. This was shown by the almost identical fractional losses of cyanocobalamin and hydroxocobalamin subsequent to the day 3 measurement.

The fractional loss of coenzyme B_{12} subsequent to the day 3 measurement was greater than that of both cyanocobalamin and hydroxocobalamin. If the natural body B_{12} is in the coenzyme form and is utilised as such, the early metabolism of vitamin B_{12} administered already in this form might be expected to differ from the other two cobalamins.

The results of this study have considerable therapeutic implications. Coenzyme B_{12} which is evidently the natural form in the body shall be discussed first. Although more coenzyme B_{12} is retained than cyanocobalamin at 3 and 28 days post administration, less is retained than hydroxocobalmin at 28 days. For this reason and because of its instability in daylight (Smith 1965) causing difficulties in processing and handling, the use of coenzyme B_{12} for routine therapy could not be justified. A pathological situation where cyanocobalamin or hydroxocobalamin could be converted to the 'in vivo' coenzyme form could warrant its use. However, such a defect has not yet been described.

In evaluating cyanocobalamin and hydroxocobalamin in the treatment of vitamin B_{12} deficiency and in calculating time-scales for intermittent therapy, parameters in addition to the amount of vitamin retained need to be considered. Allowance must be made for the individual variation in retention which is by a factor of two or more in this present series. The turnover-rate of vitamin B_{12} in patients with pernicious anaemia also varies by a factor

of two (Boddy and Adams,1968) and by more in patients with renal and hepatic disease, as shown in Chapter III. From the results of this study and by making a conservative allowance for the variations in retention and turnover, it is possible to calculate how frequently parenteral injections of 1000 µg of vitamin B_{12} will be required to maintain body stores above 1000 µg, a value which should ensure normality (Heyssel et al.,1964). The highest rate of loss observed in patients with uncomplicated pernicious anaemia was 0.2% per day. By taking the lowest value for retention at 28 days, 83 µg of cyanocobalamin and 189 µg of hydroxocobalamin, and assuming that the cobalamin loss after 28 days occurs uniformly at a rate of 0.2% per day, the calculated time between injections is 68 days for cyanocobalamin and 115 days for hydroxocobalamin. The inherent values taken are pessimistic and so the final estimated time between injections is conservative. The presence of renal or hepatic disease may affect the vitamin B_{12} metabolism and increase the cobalamin turnover-rate. The highest rate of loss observed was 0.51% per day (Chapter III) which decreases the time between injections to 44 days for cyanocobalamin and 62 days for hydroxocobalamin.

The serum vitamin B_{12} level does give an indication of the effectiveness of treatment but its value is doubtful as an index of tissue-stores in patients on intermittent therapy. For this

reason, the present calculations allowing for variation in the retention of the vitamin and the subsequent excretion rates would appear more circumspect.

SUMMARY

The retention of three parenterally administered vitamin B_{12} compounds was investigated. By 3 days after injection, the mean whole body retention of coenzyme B_{12} and hydroxocobalamin was similar and more than double that of cyanocobalamin. By 28 days, the mean retention of hydroxocobalamin was greater than that of both cyanocobalamin and coenzyme B_{12}. The fractional loss subsequent to day 3 of cyanocobalamin and hydroxocobalamin was similar and unlike that of coenzyme B_{12}.

Calculations based on the results suggest that adequate maintenance treatment of uncomplicated vitamin B_{12} deficiency will be achieved by parenteral administration of 1000 μg of cyanocobalamin every 2 months or the same dose of hydroxocobalamin every 4 months. If there is concomitant renal or hepatic disease, the same dose of cyanocobalamin should be given every $1\frac{1}{2}$ months and of hydroxocobalamin every 2 months.

CHAPTER V

ABSORPTION OF COENZYME B_{12} AND OTHER COBALAMINS AT DIFFERENT DOSE LEVELS

INTRODUCTION

The bulk of the body stores of vitamin B_{12} is in the coenzyme form (Toohey and Barker 1961, Stahlberg et al. 1967). It does not follow, however, that man's dietary intake of vitamin B_{12} is in this form. It seems likely that the extremely unstable and photosensitive coenzyme B_{12} may be converted to other cobalamins during the various preparative processes to which virtually all vitamin B_{12} containing foods are subjected. It therefore seemed of interest to measure the absorption of coenzyme B_{12} and other cobalamins in the pure form at different dose levels as a baseline for further studies.

MATERIALS AND METHODS

The absorption of radioactive coenzyme B_{12}, cyanocobalamin, hydroxocobalamin and methylcobalamin were measured each at dose levels of 1, 5 and 25 µg using a double tracer technique. Each of the 63 patients studied received the same oral dose of two cobalamins, one labelled with cobalt-57 and the other with cobalt-58 at an interval of 24 hours. The whole body activity

was measured after each dose to obtain the 100% value and again at 16 days after the first dose. All counting-rates were corrected for background, natural body radioactivity, the cobalt-58 photofraction (in cobalt-57 counting-rates) and radioactive decay.

The doses of cobalt-58 cyanocobalamin and cobalt-57 hydroxocobalamin were prepared with their respective commercially available unlabelled cobalamins. Cobalt-57 coenzyme B_{12} and unlabelled coenzyme B_{12} were prepared by the method of Johnson et al. (1963) and cobalt-58 methylcobalamin and its unlabelled form by methylation of cyanocobalamin. The doses of coenzyme B_{12} and methylcobalamin were processed in dim red light and all solutions stored at + 4°C in darkglass bottles wrapped in heavy foil. The total volume of each dose was 100 ml and the activity ranged from 0.2 μCi for the cobalt-58 cobalamins to 1.0 μCi for the 25 μg dose of the cobalt-57 cobalamins. The patients were fasting and no food was given for two hours after administration.

The subjects were hospital in or out patients with a wide variety of diseases excluding megaloblastic anaemia, malabsorption, hepatic disease and previous gastrointestinal surgery. Most were in the convalescent phase of their illness and all patients gave their informed consent to take part in the study.

RESULTS

The individual results for patients given 1 μg doses of

cyanocobalamin (CNB_{12}), coenzyme B_{12} ($CoEB_{12}$), methylcobalamin (CH_3B_{12}) and hydroxocobalamin (OHB_{12}) are presented in Table 1. Tables 2 and 3 show the results after 5 and 25 μg doses respectively. Relevant details about the subjects are also shown. The results obtained for patients given cobalt-58 cyanocobalamin and cobalt-57 coenzyme B_{12} at any one dose level and for patients given cobalt-58 methylcobalamin and cobalt-57 hydroxocobalamin at any one dose level were analysed by a 2-tailed Wilcoxon Test for matched pairs. For other comparisons a 2-tailed Mann Whitney Test for independent groups was used. A summary of these analyses is presented in Table 4.

At the 1 μg dose level the highest mean absorption was after hydroxocobalamin, the values absorbed being significantly greater than that of methylcobalamin ($P<0.01$) and coenzyme B_{12} ($P<0.02$). The values for cyanocobalamin were significantly higher than for coenzyme B_{12} ($P<0.01$) and those for methylcobalamin also significantly higher than for coenzyme B_{12} ($P<0.05$). At the 5 μg dose level, the highest mean absorption was after the administration of cyanocobalamin and the values for cyanocobalamin and methylcobalamin were both significantly greater than those for coenzyme B_{12} ($P<0.02$). At the 25 μg dose level the order changed again, the highest mean absorption being after the administration of coenzyme B_{12} and the values for both coenzyme B_{12} and hydroxocobalamin were significantly higher than those

TABLE 1

COBALAMIN ABSORPTION AT THE 1μg DOSE LEVEL

Case No.	Age	Sex	Disease	% Absorbed from dose of:		Case No.	Age	Sex	Disease	% Absorbed from dose of:	
				^{58}Co CN B_{12}	^{57}Co CoE B_{12}					^{58}Co CH_3 B_{12}	^{57}Co CH B_{12}
1	28	F	Bronchiectasis	26.4	24.5	13	60	F	Myocardial infarction	33.6	50.2
2	23	F	Rheumatic fever	52.5	40.0	14	67	F	Carcinomatosis	29.3	37.0
3	78	F	Pulmonary embolism	57.3	28.5	15	56	M	Myocardial infarction	38.2	50.4
4	47	M	Myocardial infarct	58.4	47.1	16	51	F	Purpura	43.7	40.7
5	49	F	Abdominal pain	41.5	30.8	17	61	M	Myocardial infarct	49.0	66.1
6	54	F	Cerebrovascular accident	58.1	40.6	18	41	M	Hypertension	50.0	56.2
7	64	F	Cerebrovascular accident	45.7	36.5	19	17	M	Thrombophlebitis	41.3	61.1
8	51	F	Scleroderma	41.5	33.2	20	65	F	Diabetes	59.1	71.8
9	71	F	Hiatus hernia	48.3	40.9	21	62	M	Chronic bronchitis	59.6	66.6
10	68	F	Rheumatoid arthritis	39.1	6.7	22	57	F	Obesity	40.6	57.4
11	49	F	Pulmonary embolism	36.3	46.6						
12	20	F	Obesity	84.9	29.2						
				49.2	33.7					44.4	55.7

TABLE 2

COBALAMIN ABSORPTION AT THE 5 µg DOSE LEVEL

Case No.	Age	Sex	Disease	% Absorbed from dose of: ^{58}Co CN B_{12}	% Absorbed from dose of: ^{57}Co CoE B_{12}	Case No.	Age	Sex	Disease	% Absorbed from dose of: ^{58}Co CH_3 B_{12}	% Absorbed from dose of: ^{57}Co CH B_{12}
23	61	F	Cerebrovascular accident	17.5	7.7	33	37	M	Nephritis	24.9	21.5
24	65	M	Diabetic	14.5	10.3	34	49	F	Pheumonia	13.7	10.7
25	34	F	Diabetic	13.2	11.5	35	53	F	Ulcerative colitis	14.0	18.2
26	23	M	Polyarthritis	28.5	12.2	36	48	F	Asthma	21.2	14.7
27	73	M	Diabetic	20.3	10.4	37	55	M	Myocardial infarct	9.5	12.3
28	63	M	Myocardial infarct	24.5	7.3	38	48	F	Myocardial infarct	17.3	18.5
29	72	M	Duodenal ulcer	11.5	12.5	39	37	F	Diabetic	20.7	8.9
30	64	F	Hypertension	12.2	5.7	40	53	M	Diarrhoea	11.1	10.5
31	34	F	Obesity	39.8	42.8	41	50	F	Hypertension	32.3	23.0
32	24	M	Haematemesis	21.8	8.6	42	49	F	Headache	23.6	25.0
				20.4	12.9					18.8	16.3

TABLE 3

COBALAMIN ABSORPTION AT THE 25 μg DOSE LEVEL

Case No.	Age	Sex	Disease	% Absorbed from dose of:		Case No.	Age	Sex	Disease	% Absorbed from dose of:	
				^{58}Co CN B_{12}	^{57}CoE B_{12}					^{58}Co CH_3 B_{12}	^{57}Co CH B_{12}
43	50	M	Myocardial infarct	4.0	11.3	53	41	F	Gastric ulcer	6.3	8.0
44	45	M	Diabetes	7.4	5.2	54	48	M	Duodenal ulcer	9.9	10.7
45	64	F	Cerebrovascular accident	1.3	1.9	55	43	M	Anxiety state	4.8	6.0
46	57	F	Cerebrovascular accident	5.9	5.1	56	68	M	Myocardial infarct	3.8	6.0
47	35	F	Diabetic	8.0	9.7	57	56	F	Duodenal ulcer	6.4	5.3
48	63	F	Rheumatic fever	6.5	9.6	58	71	M	Cerebrovascular accident	4.8	7.8
49	74	M	Diabetic	3.5	3.5	59	52	F	Bronchial neoplasm	10.5	9.3
50	68	F	Ischaemic heart disease	4.3	7.8	60	48	M	Cerebrovascular accident	2.9	3.2
51	64	M	Bronchial carcinoma	7.5	11.8	61	61	F	Hypertension	5.4	6.9
52	58	M	Cerebral neoplasm	7.1	12.9	62	43	M	Epilepsy	6.3	10.5
						63	52	F	Cerebrovascular accident	7.2	8.0
				5.5	7.9					6.2	7.4

TABLE 4

SUMMARY OF ANALYSES USING THE WILCOXON TEST FOR MATCHED PAIRS AND THE MANN WHITNEY TEST FOR INDEPENDENT GROUPS

1 µg DOSE

	MEAN % ABSORPTION
OHB_{12}	55.7
CNB_{12}	49.2
CH_3B_{12}	44.4
$CoEB_{12}$	33.7

	CN	CoE	CH_3	OH
CN		S^{+++}	NS	NS
CoE			S^{+}	S^{++++}
CH_3				S^{+++}
OH				

5 µg DOSE

CNB_{12}	20.4
CH_3B_{12}	18.8
OHB_{12}	16.3
$CoEB_{12}$	12.9

	CN	CoE	CH_3	OH
CN		S^{++}	NS	NS
CoE			S^{++}	NS
CH_3				NS
OH				

25 µg DOSE

$CoEB_{12}$	7.9
OHB_{12}	7.4
CH_3B_{12}	6.2
CNB_{12}	5.5

	CN	CoE	CH_3	OH
CN		S^{+}	NS	S^{+}
CoE			NS	NS
CH_3				NS
OH				

NS - result not significant $P > 0.05$

S - result significant

\+ $P < 0.05$

++ $P < 0.02$

+++ $P < 0.01$

++++ $P < 0.002$

for cyanocobalamin ($P<0.05$).

DISCUSSION

On the assumption that the whole body radioactivity 16 days after oral administration of a labelled cobalamin is a measure of the amount of cobalamin absorbed, the results seem to indicate that the fraction of the dose absorbed is a function of both the mass and the structure of the cobalamin. The effect of mass on the fraction of the dose absorbed has been shown by Glass et al. (1954), who found the oral absorption of vitamin B_{12} in normal subjects to vary from 3% after 50 µg of cyanacobalamin to 40% after 2 µg and by Swendseid et al. (1954) who found it to vary from 16% after 10 µg of cyanocobalamin to 68% after 0.5 µg. The nature of the ligand occupying the 6th place on the cobalt atom determines the cobalamin structure. This was found to be important by Rosenblum et al. (1955, 1956), cyanocobalamin being better absorbed than chlorocobalamin, sulphitocobalamin, nitrocobalamin and thiocyanatocobalamin at dose levels of 0.5 to 2.0 µg. Structure was also shown to be a factor by Herbert and Sullivan (1964) who found that cyanocobalamin was better absorbed than coenzyme B_{12} at the 2.0 µg dose level and by Heinrich and Gabbe (1964) who showed that the absorption of cyanocobalamin and hydroxocobalamin was similar and greater than that of coenzyme B_{12} at 0.1 to 1.5 µg dose levels. The interrelation-

ship of mass and structure with respect to the fraction absorbed has not however been previously described.

An explanation for the different cobalamin fractions absorbed at different dose levels, or even at any one dose level, is not apparent. The cyano group in cyanocobalamin is so tightly bound that the vitamin B_{12} is essentially a non-electrolyte. Other cobalamins, such as hydroxocobalamin or chlorocobalamin, are highly dissociated so as to be effectively electrolytes in character. Rosenblum et al. (1955) suggested that this difference in behaviour might explain why some cobalamins are better absorbed from the gastrointestinal tract than others. However, Rosenblum et al. (1956), subsequently concluded that the differences in the ease of absorption of cobalamins could not be attributed to differences in their electrolyte nature and attendant solubility properties and thought that the tightness of binding of the cyano group in the cobalt co-ordination sphere accounted for the better absorption of cyanocobalamin compared with chlorocobalamin, sulphitocobalamin, nitrocobalamin and thiocyanatocobalamin at least at the 2 µg dose level. This explanation would be in keeping with our results at the 1 and 5 µg dose levels but not with those at the 25 µg level. There is evidence, however, that the absorption of at least cyanocobalamin is intrinsic factor independent at the 30 µg dose level (Herbert et al. 1964) and it may be that factors such as tightness of binding of the ligand are

important when the absorption is intrinsic factor dependent, but are less so, or compensated for, when the absorption is independent of the intrinsic factor mechanism. The degrees of significance for the difference in the absorption of one cobalamin compared with another (Table 4) appear to support this suggestion. In general, they are high at the 1 μg dose level and become less impressive with increased doses, until, after the 25 μg doses, the degrees of significance only just reach the 5% level.

Whether these findings have any physiological significance is uncertain. It has been shown that the absorption of meat bound cobalamin was comparable with that of pure cyanocobalamin (Heyssel et al. 1966) and although Reizenstein and Nyberg (1959) found that liver bound cobalamin is more readily absorbed than pure cyanocobalamin this finding was not confirmed by Sullivan et al. (1962) or Okuda et al. (1968). Nevertheless, extrapolation from the present relatively simple unphysiological study to the complex field of absorption from food would seem unwise. It can be concluded from the results, however, that the absorption of vitamin B_{12} appears to be an increasingly complex subject.

SUMMARY

The absorption of coenzyme B_{12} and other cobalamins in pure form at different dose levels has been studied in 63 patients

using a double tracer technique. The fraction of the dose absorbed appears to be a function of both the mass and the structure of the cobalamin. It may be that factors which account for differences in absorption when the mechanism is intrinsic factor mediated differ from those when absorption is intrinsic factor independent. The study confirms the complexity of the absorption of vitamin B_{12}.

CHAPTER VI

A PRELIMINARY STUDY OF ZINC METABOLISM IN CARCINOMA OF THE PROSTATE GLAND

INTRODUCTION

Zinc is a trace metal of physiological importance which is widely distributed in many enzyme systems. Changes in the activity of certain enzymes, depending on the presence of specific trace metals, have been reported in tumour tissues of animals (Greenstein 1954) and of man (Dacha et al. 1963). The remarkably high concentration of zinc in the human prostate was first shown by Bertrand and Vladesco (1921) and has since been well established (Mawson and Fischer 1951, Gunn et al. 1955, Vallee 1959, McKenzie et al. 1962, Mayer 1964). When a carcinomatous change occurs in the prostate there is a distinct fall in the zinc content of the prostatic tissue (Mawson and Fischer 1952, Hoare et al. 1956, Daniel et al. 1956, Schrodt et al. 1964, Rosoff and Spencer 1965, Gyorkey et al. 1967).

These observations suggest that a comparison of whole body zinc metabolism related to local prostatic uptake of zinc might form the basis of a method to measure the response of prostatic carcinoma to therapy.

Preliminary investigations have been carried out to establish the sources of error and to optimise the procedures (Boddy et al. 1970 B).

MATERIALS AND METHOD

Five male patients (cases 1 to 5) between 60 and 70 years of age who were at various stages of oestrogen therapy for prostatic carcinoma were investigated. A male control patient (case 6) who underwent total cystectomy for bladder tumour and was not known to have any prostatic lesion was included in the study.

Each patient was given an intravenous dose of 10 μCi zinc-65 as zinc chloride and a whole body measurement was made within two hours of administration. Surface counting was carried out over the perineum using a 7.6 by 7.6 cm sodium iodide detector with a lead collimator. Whole body and surface measurements were made up to 6 to 8 days post administration and up to 15 days in case 2. Direct counting of the right and left lobes of the prostate was achieved using a new rectal probe (Boddy and Provan, 1970) which was directed against each lobe of the gland in turn by a finger placed in the rectum. Rectal counts were made and blood was withdrawn twice daily for four consecutive days. The blood was centrifuged at 6,000 r.p.m. for ten minutes and after separation, stored at a temperature of - 4°C. All patients were

on 24 hour urine and faecal collections for 4 days.

RESULTS

The clinical data for the patients studied are given in Table 1.

Figure 1 shows the whole body retention of zinc - 65. The maximum variation in counting-rate up to about 8 hours post administration did not exceed 2%, typical results for three of the patients being shown in Chapter II, Table 7 and subsequent variation up to 24 hours post administration was less than 4%. It was intended that each patient should receive the same dose of 10 μCi zinc - 65. As differences in the initial counting-rates among individuals greatly exceeded those seen previously with a wide range of isotopes, it seems likely that despite the care taken, the amount of zinc - 65 administered was variable. Consequently, comparison with standards was not very meaningful and, where appropriate, results were normalised to the initial whole body counting-rates.

The results of the 24 hour urine and faecal collections, given in Table 2, show that the mean loss up to 4 days post administration is 4.4% and that the bulk of the zinc - 65 is lost in the faeces.

A geometry correction factor was applied enabling an approximate comparison to be made with the retention figures

TABLE 1

CLINICAL DATA

CASE NO.	AGE (1968)	DIAGNOSIS	DATE	HISTOLOGY OF PROSTATE	HORMONE THERAPY	DOSE
1	68	PROSTATIC CARCINOMA	27.5.62	ANAPLASTIC ADENO-CARCINOMA: EXTENSIVE INFILTRATION OF TISSUE	TACE 27.5.62- 6.1.64 HONVAN 10.7.68-15.7.68 16.7.68-21.7.68 22.7.68-	12 mg t.i.d. 500 mg i.v. 100 mg i.v. 100 mg t.i.d.
2	62	PROSTATIC CARCINOMA (TRANSURETHRAL RESECTION)	22.9.70	MOD. WELL DIFFERENT-IATED ADENOCARCINOMA: INFILTRATING PERI-LYMPHATIC TISSUE	STILBOESTROL 22.9.67-21.1.68 27.1.68-	15 mg t.i.d. 5 mg t.i.d.
3	64	PROSTATIC CARCINOMA	20.9.68	TWO ADENOCARCINOMATOUS FOCI, REST BENIGN HYPERPLASIA	STILBOESTROL 5.10.68-	10 mg t.i.d.
4	66	PROSTATIC CARCINOMA	7.11.67	NOT AVAILABLE	STILBOESTROL 7.11.67-4.10.68 4.10.68-	20 mg t.i.d. 10 mg t.i.d.
5	64	PROSTATIC CARCINOMA WITH SECONDARY DEPOSIT IN HUMERUS	18.8.67	'SUGGESTION' OF ADENO-CARCINOMA ON NEEDLE BIOPSY	STILBOESTROL 18.8.67-	10 mg t.i.d.
6	54	CARCINOMA OF BLADDER	2.10.68	ADENOMATIONS HYPERPLASIA	-	-

TACE = TRIPARA-ANISIL-CHLORETHYLENE

HONVAN = DIETHYLSTILBOESTROL DIPHOSPHATE

FIG I RETENTION OF ZINC - 65 ESTIMATED BY WHOLE - BODY MONITORING

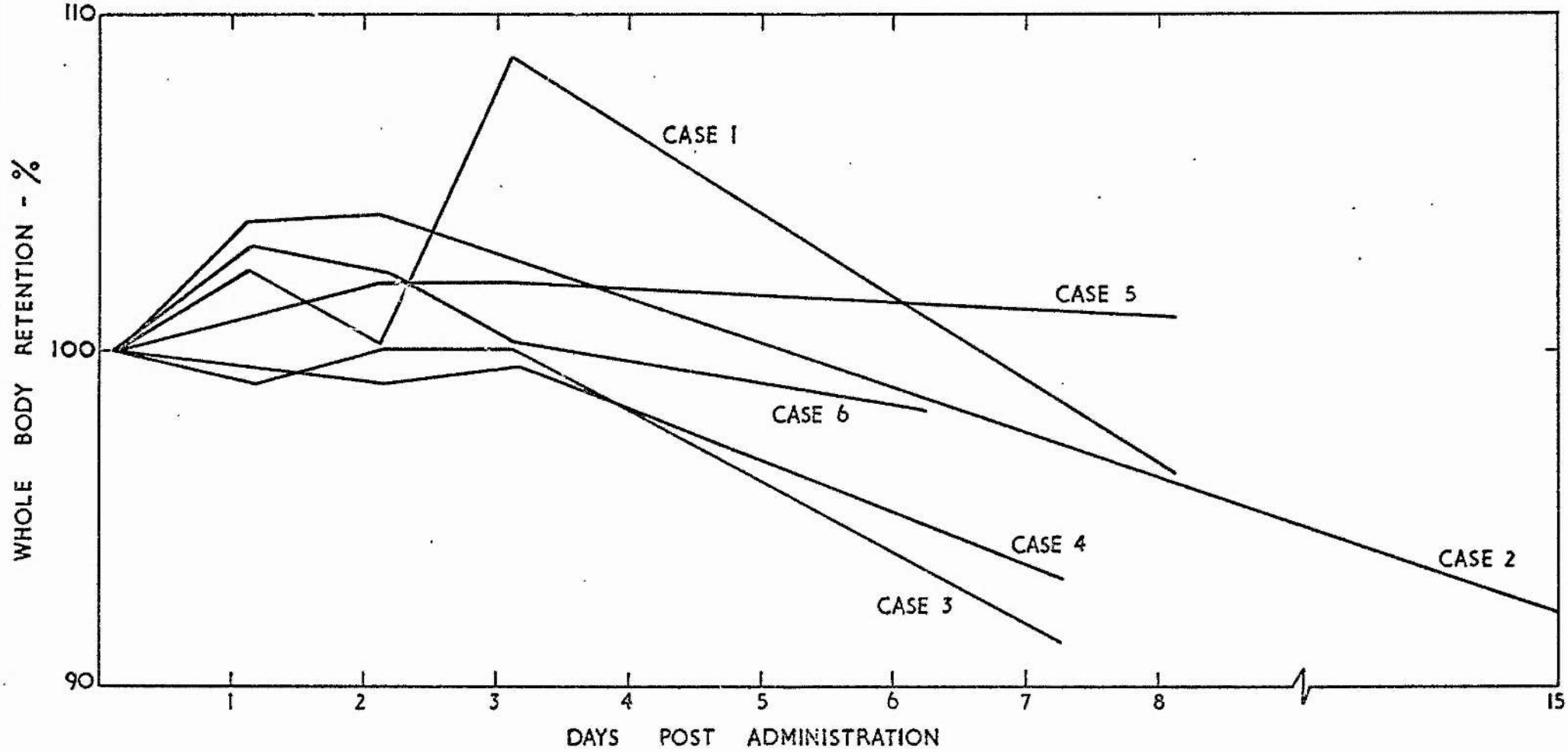


TABLE 2

EXCRETION OF ZINC - 65 IN URINE AND FAECES AS % OF DOSE

CASE No.	DAYS								TOTAL DAYS 0 - 4 (U+F)
	0 - 1		1 - 2		2 - 3		3 - 4		
	U	F	U	F	U	F	U	F	
1	0.46	0.49	0.44	0.25	0.25	1.04	0.27	0.01	3.21
2	0.09	0.37	0.11	1.04	0.04	-	0.04	0.72	2.41
3	0.36	-	0.29	1.57	0.03	0.25	0.24	2.09	4.83
4	0.88	0.04	3.74	0.18	0.38	0.28	0.26	0.75	6.51
5	1.15	-	0.46	1.25	0.43	1.93	0.26	-	5.48
6	0.15	0.01	0.20	1.04	0.12	0.67	0.11	1.68	3.98
MEAN	0.52	0.15	0.87	0.89	0.21	0.70	0.20	0.88	4.40

U - URINE

F - FAECES

calculated from the whole body measurements. Reasonable agreement between the two methods was obtained, with the exception of one unexplained whole body result for case 1 on day 3.

The probe and surface counting results are shown in Figures 2 and 3 respectively. The limited sensitivity of the prototype probe may account for some of the results which were not significantly different from background. However, the findings for individual patients were fairly constant. The rectal probe measurements showed a consistently higher prostatic uptake of zinc -65 in cases 2 and 5 and case 2 also gave the highest surface counting results. With long term follow-up of the patients, the clinical significance of these findings may become clear.

The zinc - 65 levels in red cells and plasma, normalised for each individual to their initial whole body count are shown in Figure 4. The high specific activity (μCi / ml) of zinc - 65 in the red cells can be seen.

DISCUSSION

The diagnosis of prostatic carcinoma and the determination of the clinical progress of the disease are mainly dependent on rectal examination, a technique that has certain limitations. The use of prostatic biopsy by the transrectal or transperineal method has the limitation of probably missing a small focus of

FIG 2 RESULTS OF RECTAL PROBE NORMALISED TO WHOLE-BODY RETENTION

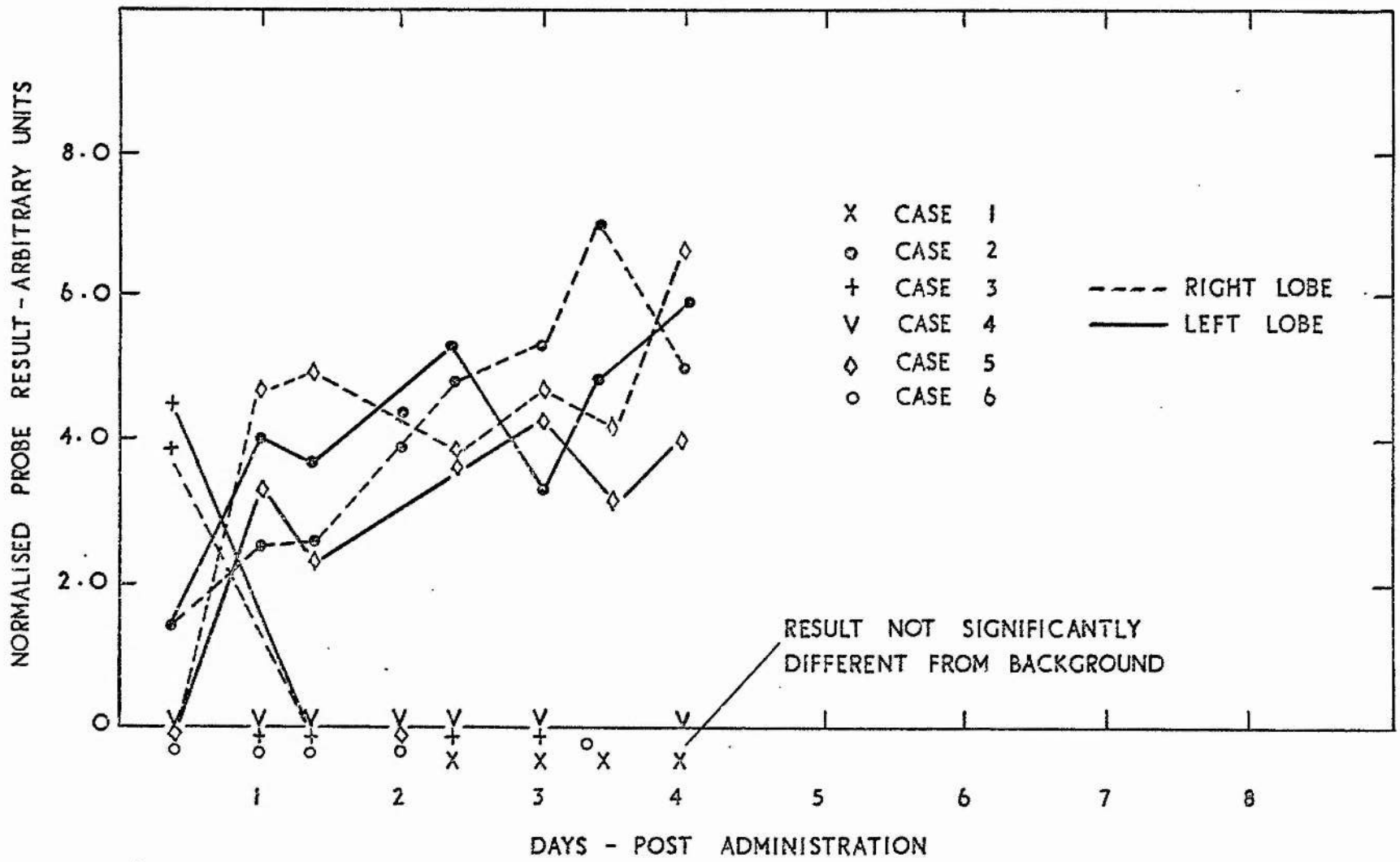


FIG 3 SURFACE COUNTS NORMALISED TO WHOLE-BODY RETENTION

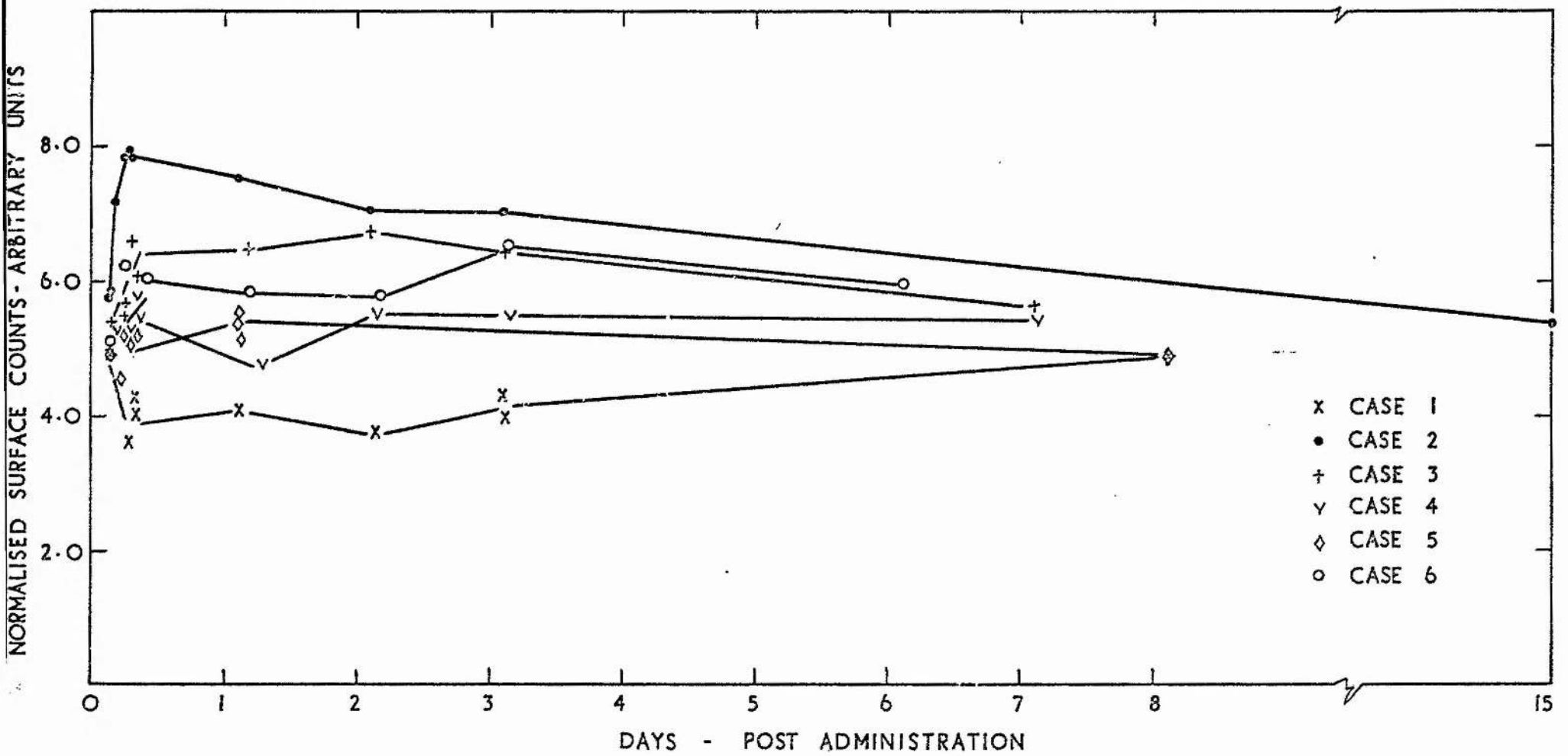
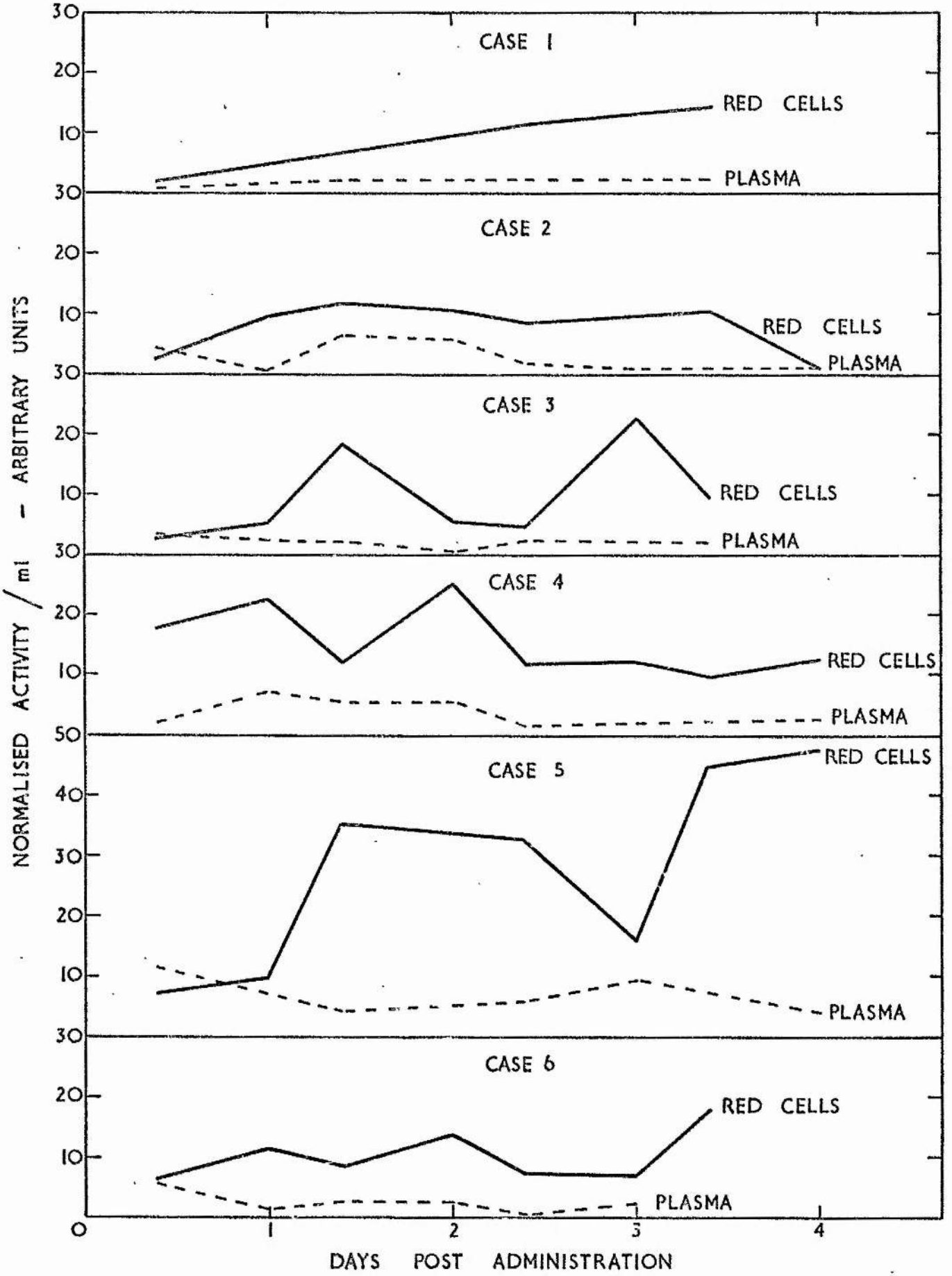


FIG 4 ZINC-65 IN RED CELLS AND PLASMA NORMALISED TO INITIAL WHOLE-BODY COUNT



tumour and the possible introduction of infection into an otherwise sterile organ. In some cases of prostatic carcinoma there is an elevation of the serum phosphatase (Nesbit and Baum 1951, Morgan and Mills 1968), but this relatively simple biochemical estimation does not afford a constant method of monitoring the clinical progress of the disease. Similarly, there may be an increase in urinary enzyme output but again this has proved unreliable in studies of prostatic carcinoma, since only about 30% of the cases show an increase in urinary output of alkaline phosphatase and only about 50% of the cases an elevation in the output of lactic acid dehydrogenase (Gault et al. 1967).

Zinc offers a possible additional method of studying adenocarcinoma of the prostate and the clinical effectiveness of treatment because of its high specific affinity for prostatic tissue (Kar and Chowdhury,1966) and its inclusion in the metalloenzyme complexes. The uptake of zinc by the prostate is hormone dependent (Gunn and Gould 1956, McKenzie et al. 1963, Kar and Chowdhury 1966) and a study of the zinc metabolism would enable the response of different tumours to different oestrogens to be determined. This lends a further attraction to the use of zinc in studying prostatic carcinoma.

Zinc is not uniformly distributed throughout the prostate gland, the dorsolateral zone of the rat prostate being the area

of greatest zinc concentration (Gunn et al., 1955). Gyorkey et al. (1967) reported that in the normal human prostate zinc is similarly distributed, the dorsal and more so the lateral zones having a higher zinc level than the other areas of the gland. These authors (Gyorkey et al., 1967) have also shown that, in the carcinomatous gland, there may be up to a 78% decrease in the zinc concentration in the dorsolateral zone and a 50% decrease in the inner or anterior zone. The dorsolateral zone of the prostate, the area where a neoplasm often arises, is the most accessible for rectal counting and will also be the area of maximum change in zinc concentration.

The present preliminary study showed that the overall investigatory procedures were satisfactory and where modifications and improvements could be made. Spencer et al. (1966) have shown that at least 17 days is required for peak zinc uptake by the prostate and it was anticipated that the duration of the present study would be inadequate. The loss of zinc - 65 was measured for the first 4 days of the study by both the whole body monitor and by the assay of urine and faecal collections. The loss was shown to be small (mean of 4.4%) and primarily in the faeces. Collection and assay of excreta over a longer period would be impracticable and probably inaccurate, and since the excreta results essentially substantiate the findings with the whole body monitor, collections

will not be made in future studies. The performance of the monitor was considered satisfactory with respect to variations in the counting-rates and precision. The maximum variation up to 12 hours post administration was less than 2% and up to 24 hours was less than 4%. Only one result, unaccountably, was subsequently outside these limits. The whole body measurements also proved of value because of difficulties in dispensing the zinc - 65. The solution was accurately dispensed by volume, but for reasons still being investigated the amount of solution administered was variable. In further studies the syringes will be monitored before and after administration.

The size of the detector, 1.0 cm in diameter by 1.0 cm in length, used in the rectal probe was inconveniently large. To permit accurate location of the probe against each lobe of the prostate, the performance of a smaller detector is being investigated. Further development is expected to reduce the variation in sensitivity which was troublesome with the prototype probe and resulted in a number of readings not significantly different from background counting-rates.

The principal difficulty in surface counting arose from the comparatively large uptake of zinc - 65 by the liver resulting in a significant contribution to the counting-rate over the prostate. More sophisticated collimation is expected to reduce this problem.

The possible use of zinc - 65 and zinc - 69m in scinti-scanning of the prostate has been investigated (Johnston et al. 1968) and reasonable scans have been obtained. Verilly et al. (1962) had difficulty in obtaining scans of prostatic tissue but these authors did suggest that better scans would be obtained if their collimation could be improved. It is intended in future studies to use scintigraphy which should provide additional information, possibly indicating the size and position of lesions.

The present findings suggest that the methods described, with the improvements outlined, will be useful in following the clinical progress of prostatic carcinoma and its response to hormone therapy.

SUMMARY

The human prostate gland contains a remarkably high concentration of zinc which, when a carcinomatous change occurs in the gland, becomes considerably diminished. It is suggested that a study of the whole body metabolism and the local prostatic uptake of zinc - 65 may provide the basis of a method for measuring the response of prostatic carcinoma to therapy. Preliminary investigations have been carried out on 5 patients with adenocarcinoma of the prostate and on 1 control subject to determine the practicability of the procedures and where

modifications and improvements could be made.

CHAPTER VII

ANAEMIA IN CHRONIC RENAL FAILURE

INTRODUCTION

The occurance of anaemia in patients with chronic renal failure and in patients undergoing regular dialysis treatment is well established and in both these groups the metabolism of iron is known to be abnormal. Malabsorption of iron, impaired erythropoiesis, excessive iron losses due to the loss of blood or increased urinary excretion of iron are possible causes of this. Kay (1958) and Loge et al. (1958) have shown the rate of erythropoiesis to be depressed in patients with chronic renal failure, resulting in a decreased red cell incorporation of iron (Ragan et al., 1960). These factors have been further investigated in a comprehensive study with particular reference to the oral absorption of iron and to the iron turnover rates (Boddy et al., 1970 A). These two parameters have not previously been well established. The results for patients with chronic renal failure not on dialysis therapy are given in SECTION I and for patients with chronic renal failure on regular dialysis therapy in SECTION II.

The anaemia of chronic renal failure is not corrected by regular dialysis treatment and until recently, it was thought

that repeated blood transfusions should be given to maintain haemoglobin levels (Eschbach et al., 1967). Several workers have now reported that patients on regular dialysis therapy have been maintained for long periods without blood transfusion (Comty et al. 1966, Crockett et al. 1967, Verroust et al. 1967). Apart from the consequent reduction in the cost of treatment, this would also lessen the risk of serum hepatitis in patients and staff. If routine blood transfusion is to be avoided, the major sources of blood loss must be found and minimised. Blood removed for routine haematological investigations, which can easily be minimised, and blood left in the artificial kidney after dialysis are the two main sources of loss. Evidence is now accumulating to suggest that patients on regular dialysis treatment may become iron deficient (Shaldon 1966) and that this may be due to blood losses occuring mainly in the artificial kidney (Lawson et al., 1968). The study described here includes an extensive investigation of the blood losses in a Kolff twin-coil artificial kidney to enable the optimum operating procedures for minimal losses to be determined (Will et al., 1970). The effect of varying coil pressure, coil position and the volume and composition of the wash-back fluid on coil blood loss have been examined. The results are given in SECTION II.

SECTION I

IRON METABOLISM IN PATIENTS WITH CHRONIC RENAL FAILURE NOT RECEIVING DIALYSIS THERAPY

MATERIALS AND METHODS

Nineteen patients, 11 females and 8 males, with chronic renal failure of 2 to 4 years' duration were included in the study. Two patients were suffering from polycystic disease, the remainder having end-stage renal failure. No patient had active infection at the time of the study and eighteen were taking a modified Giovannetti diet (Shaw et al., 1965). All patients had creatinine clearances of 10 ml per minute or less, and during the course of the study were in a steady state with respect to renal function.

Twelve of the patients fasted overnight and after background radioactivity measurements, were given oral doses of 5 mg ferric iron labelled with 1.0 μCi iron-59, made up to a total volume of 150 ml with water. After taking the dose, the subjects fasted for a further hour. Measurements of the whole body iron-59 radioactivity were made at 1 hour and 20 days after administration, the percentage absorption of iron being calculated after appropriate corrections for patient background and radioactive decay.

Following background radioactivity measurements, 10 patients

were given an intravenous dose of 1.0 μCi iron-59. The 100% retention was measured on the day of administration and then sequentially up to 308 days. Exponential excretion rates were calculated by fitting regression lines using the method of least squares. The percentage incorporation of iron-59 was estimated from blood samples drawn 10 days after administration.

RESULTS

The oral iron absorption results together with the patients clinical data can be seen in Table 1. In all cases the Coomb's test was negative, the serum bilirubin levels were normal and in no patient was the average reticulocyte count greater than 4%. The mean iron absorption and standard error was 3.47 ± 0.90%. There was no significant difference ($P > 0.5$) between the value for males only, 2.45 ± 1.25%, and for females only, 3.98 ± 1.21%. In control subjects (Will and Boddy, 1967), the mean iron absorption and standard error in all subjects was 10.00 ± 0.85%, 9.56 ± 0.91% in males only and 11.40 ± 2.19% in females only. The difference between the mean iron absorption in patients with chronic renal failure and in normal controls was found to be highly significant ($P < 0.001$).

The clinical data, the excretion rates and red cell incorporation of iron-59 for the patients given intravenous iron can be seen in Table 2. The mean excretion rate of iron-59 and

TABLE 1

ORAL IRON ABSORPTION IN PATIENTS WITH CHRONIC RENAL FAILURE TOGETHER WITH THEIR RENAL AND HAEMATOLOGICAL DATA

Patient	Sex	Age	Diet	Creatinine Clearance ml/minute	Urea mg %	Hb g %	M.C.H.C. %	Fe µg %	T.I.B.C. µg %	% Sat'n	Fe Absorption %
1	M	64	40 g	7	170	10.6	33	80	274	29.2	5.8
2	M	62	40 g	6	136	11.1	35	-	-	-	2.8
3	M	27	40 g	6	128	15.0	35	60	166	36.1	1.1
4	M	45	40 g	4	96	9.8	33	66	288	22.9	0.1
5	F	48	Normal	10	130	15.3	34	40	220	18.2	5.2
6	F	60	40 g	10	94	11.7	33	48	343	14.0	0.1
7	F	39	40 g	9	100	10.8	35	-	-	-	10.3
8	F	67	18 g	7	138	12.4	34	33	219	15.1	6.3
9	F	62	18 g	6	121	9.7	34	120	342	35.1	0.4
10	F	36	18 g	6	125	5.4	32	40	247	16.2	5.0
11	F	45	18 g	4	129	6.4	31	42	232	18.1	2.2
12	F	50	18 g	4	150	6.9	34	120	252	47.6	2.3

TABLE 2

LONG-TERM IRON TURNOVER AND IRON INCORPORATION IN PATIENTS WITH CHRONIC RENAL FAILURE TOGETHER WITH THEIR RENAL AND HAEMATOLOGICAL DATA

Patient	Sex	Age	Diet	Creatinine Clearance ml/minute	Urea mg %	Hb g %	M.C.H.C. %	Fe µg %	T.I.B.C. µg %	% Sat'n	Fe excretion Rate % per day	Fe Incorporation %
3	M	27	40 g	6	128	15.0	35	60	166	36.1	0.10	88
7	F	39	40 g	9	100	10.8	35	-	-	-	0.40	40
10	F	36	18 g	6	125	5.4	32	40	247	16.2	0.31	29
13	F	54	40 g	9	114	14.0	32	102	420	24.3	0.24	62
14	F	48	18 g	5	200	8.9	32	44	264	16.7	0.17	45
15	F	37	18 g	10	74	9.8	31	-	-	-	0.18	43
16	M	46	40 g	10	160	14.1	32	42	194	21.7	0.13	42
17	M	44	18 g	3	134	5.2	32	50	227	22.0	0.07	40
18	M	19	18 g	3	130	7.4	33	48	366	13.1	0.07	45
19	M	23	18 g	5	100	6.4	31	66	328	20.1	0.09	17

the standard error was 0.18 ± 0.04% per day, the individual values ranging from 0.07 to 0.40% per day. The excretion rates in control subjects ranged from 0.04 to 0.26% per day with a mean and standard error of 0.15 ± 0.04% per day (Will and Boddy, 1967). The two mean excretion rates were not significantly different ($P>0.05$). The percentage red cell iron incorporation ranged from 17 to 88% with a mean and standard error of 45.1 ± 6.0% , whereas, in control subjects it ranged from 73 to 90% with a mean and standard error of 81.0 ± 1.4%. Patients with chronic renal failure showed a significantly lower red cell incorporation of iron-59 ($P<0.02$).

DISCUSSION

Although 6 of the 19 patients were severely anaemic with haemoglobin levels of less than 8g per 100ml of blood and a further 7 were moderately anaemic as judged by generally accepted normal haematological values (Dacie and Lewis, 1968), the iron-59 absorption study showed a diminished oral iron uptake in these patients. Eighteen of them were taking a modified Giovannetti diet which is known to have an iron content of 8 to 12 mg per day (Shaw et al., 1965) compared with the normal dietary iron content of 12 to 15 mg per day (Price, 1963). The diminished absorption of oral iron coupled with prolonged marginally sub-normal dietary intake of iron would seem to suggest that iron

deficiency might be the cause of the anaemia. Another possible way in which iron deficiency could develop in these patients was shown by Cartwright et al. (1954) and Dagg et al. (1966) who found excessive urinary iron losses in association with proteinuria. The serum iron values seem also to support the suggestion of iron deficiency, 12 of the 16 cases examined having serum iron levels below 80 µg per 100 ml and 7 having transferrin saturation values of less than 20%.

However, there is evidence contra-indicating iron deficiency as the cause of the anaemia. The serum iron level and the percentage transferrin saturation were shown by Weinfeld (1965) to be unreliable indicators of the body iron content in patients who are suffering from conditions other than iron deficiency. This was also shown by Curtis et al. (1969) on a group of patients with chronic renal failure on regular dialysis therapy, particularly when the serum iron was below 100 µg per 100 ml. Eight of the 10 patients investigated had a normal iron excretion rate showing the urinary iron loss in association with proteinuria was not abnormal. The 2 patients in whom the iron excretion rate was greater than normal had obvious extra-renal sources of blood loss. However, none of the patients had a daily protein loss in excess of 0.5 g which is apparently much less than the protein loss of 6 g daily or more, at which level proteinuria might be a source of body iron loss. Further evidence against iron

deficiency being the cause of the anaemia was the normal mean corpuscular haemoglobin content which was found in all patients. None of the peripheral blocd films showed evidence of hypochromia or microcytosis. It seems unlikely therefore, that the anaemia which is both normochromic and normocytic is caused by iron deficiency.

Normal subjects on a restricted dietary iron intake show an increase in the percentage of iron absorbed (Pollack et al., 1964). The patients with chronic renal failure however showed a decrease in iron absorption. As mentioned earlier, these patients are known to have a depressed erythropoietic rate which presented in a decreased red cell incorporation of iron. The diminished absorption of oral iron could result from the depression of erythropoiesis since this is a factor which influences the absorption of iron (Bothwell et al. 1958, Crosby 1963, Price 1963, Wheby 1966). There was no clinical evidence of increased haemolysis and all patients had normal serum bilirubin levels, negative direct Coomb's test and a normal reticulocyte count.

Although the absorption of iron is decreased in patients with chronic renal impairment and for prolonged periods their dietary iron intake is sub-optimal, there is no evidence to suggest that iron deficiency plays a significant part in the aetiology of the anaemia.

SECTION II

IRON METABOLISM IN PATIENTS WITH CHRONIC RENAL FAILURE ON REGULAR DIALYSIS THERAPY

MATERIALS AND METHODS

The absorption of oral iron and the iron turnover rates were investigated in 11 patients with chronic renal failure, who were receiving regular dialysis therapy using a Kolff twin-coil dialyser. All patients had severe chronic renal failure secondary to either chronic glomerulonephritis, chronic pyelonephritis or polycystic disease of the kidneys. All had creatinine clearance levels of 4 ml per minute or less and were being maintained on 10 hour dialyses twice or thrice weekly, using both Chron-a-coils and Ultra-flo 100 coils (Baxter-Travenol). No patient received oral iron supplement during the time of the study. The oral absorption of 5 mg of ferric iron labelled with 1.0 μCi iron-59 was measured in 5 patients using the method described in Section I. Following background radioactivity measurements, 8 of the patients were given 1.0 μCi iron-59 intravenously. The 100% retention was measured on the day of administration and then, in 6 of the subjects, sequentially up to 260 days post-administration. The excretion rates were calculated as in Section I. Blood samples were withdrawn from the 8 subjects 10 days after administration and

the percentage incorporation of iron-59 into the red blood cells estimated.

The amount of blood left in the artificial kidney after dialysis has been measured using both Chron-a-coils and Ultra-flo 100 coils. Blood loss in the coils after wash-back were measured by one of three methods:-

1. The coils were immediately washed out with 1 litre of normal saline and the washings thoroughly mixed. The haemoglobin and the packed cell volume of this fluid was then determined using the Cyan-methaemoglobin method and a microhaematocrit. Knowing the haemoglobin and the packed cell volume of the patient's blood, the volume of the blood lost in the coil could then be calculated.

2. A sample of 20 ml of patient's blood was removed and the red cells labelled with radio-chromium (Mollinson, 1967). This was then re-injected into the patient and time allowed for equilibration to take place. The coils from subsequent dialyses were sealed in polythene bags and replaced in their cardboard containers before counting in the whole body monitor in a standard geometry with respect to the detector. Six measurements were made on each coil, each face of the container being counted once and the mean result taken as the final figure. A standard was made by filling a coil identical to the test one with a solution containing a known volume of patient's blood withdrawn at the

beginning of the test dialysis. The standard and test coils were counted in the same geometry. Knowing the amount of blood in the standard coil, the residual blood volume in the test coil could be calculated.

3. Patients were given an intravenous dose of 1.0 μCi iron-59 and time allowed for the incorporation of the labelled iron into the red blood cells. Coils from subsequent dialyses were counted in the whole body monitor in a standard geometry. The coils were then washed out with normal saline and recounted. The washings were made up to a standard volume of 500 ml and counted. A known volume of the patient's blood, withdrawn at the beginning of the test dialysis, was diluted to 500 ml with normal saline and counted in the same geometry as the washings. This made it possible to measure accurately the amount of blood washed out of the coil after its initial counting. This blood volume represented the difference between the first and second coil counts. The total blood loss in the test coil could then be calculated.

Routine wash-back technique:

At the end of the dialyses, the arterial side of the patient's shunts were closed and blood in the arterial tubing of the kidney allowed to enter the coils. The wash-back fluid which was pumped into the arterial side of the coils at a flow rate of 150 ml per minute flushed the blood through the coils and back into the

patient's body at the venous side. During the wash-back the coils were lifted from the containers and placed on their side above it to facilitate the drainage of blood. The Ultra-flo 100 coils were not maintained within the air cuff during wash-back but lifted free of this before being placed on their side on top of the container. At no time was air introduced into the system since the risk of an air embolus reaching the patient might be greatly increased.

RESULTS

The haematological data, the oral absorption, red cell incorporation and excretion rate of iron-59 can be seen in Table 3. In all cases the Coomb's test was negative, the serum bilirubin levels were normal and in no patient was the average reticulocyte count greater than 4%. The mean iron absorption and standard error was 1.08 ± 0.24% compared with 3.47 ± 0.90% in patients with chronic renal failure not on dialysis and 10.00 ± 0.85% in normal control subjects (Will and Boddy, 1967). The mean absorption in patients on dialysis was significantly less than in normals ($P<0.001$), but was not significantly less than in patients with chronic renal failure not on dialysis ($P = 0.06$).

The percentage red cell iron incorporation ranged from 12 to 59% with a mean and standard error of 31.0 ± 5.7% compared

TABLE 3

ORAL IRON ABSORPTION; LONG-TERM IRON TURNOVER AND IRON INCORPORATION IN PATIENTS WITH CHRONIC RENAL FAILURE ON REGULAR DIALYSIS THERAPY TOGETHER WITH THEIR HAEMATOLOGICAL DATA

PATIENT	SEX	AGE	Hb g %	M.C.H.C. %	SERUM Fe µg %	T.I.B.C. µg %	% SAT'N	ORAL ^{59}Fe ABSORPTION	R.B.C. ^{59}Fe INCORPORATION	EXCRETION RATE % PER DAY
1	M	19	6.1	33	44	368	12.0	-	47	0.41
2	M	-	6.8	31	35	175	20.0	1.6	17	0.54
3	M	39	6.2	35	43	561	7.7	-	27	0.43
4	M	37	5.8	34	48	464	10.6	0.2	59	-
5	M	27	6.2	31	12	385	3.1	-	12	0.72
6	M	44	7.0	33	24	493	4.9	-	40	1.46
7	M	24	6.3	34	138	240	57.6	1.0	-	-
8	M	15	5.1	33	68	410	16.5	-	22	-
9	F	36	6.1	33	75	356	21.1	1.5	24	-
10	F	45	5.8	33	32	389	8.2	1.1	-	-
11	F	17	5.7	32	35	372	9.4	-	-	0.73

with 45.1 ± 6.0% in patients with chronic renal failure not on dialysis and 81.0 ± 1.4% in normal subjects (Will and Boddy, 1967). The mean incorporation of iron-59 in patients on dialysis was significantly less than in normals ($P<0.01$), but was not significantly less than in patients with chronic renal failure not on dialysis ($P>0.05$).

The mean excretion rate of iron-59 and the standard error was 0.73 ± 0.13% per day, the individual values ranging from 0.41 to 1.46% per day. The mean excretion rate and standard error in patients with chronic renal failure not on dialysis was 0.18 ± 0.04% per day and in normal controls was 0.15 ± 0.04% per day. Patients on dialysis therapy had a significantly greater excretion rate ($P<0.001$) than patients with chronic renal failure not on dialysis and normal controls.

Blood losses in 7 Chron-a-coils and 8 Ultra-flo coils were examined using the haemoglobin and packed cell volume method (method 1). The blood losses were calculated by measuring the volume of the washings (V), the packed cell volume of the washings (PW) and the packed cell volume of the patients blood (PP).

$$\text{Then the blood loss} = \frac{V \times PW}{PP} \text{ ml}$$

This result was then checked using the haemoglobin instead of the packed cell volume. Using this method, blood losses appeared to be 30 to 50 ml per Chron-a-coil and 15 to 30 ml per Ultra-flo coil. However, for reasons which will be discussed

later, this method was considered so inaccurate as to be almost meaningless and no further work was done using the technique.

Eight Chron-a-coils and 3 Ultra-flo 100 coils were examined using the radio-chromium method (method 2). In order to obtain a realistic estimate of the blood losses, the blood remaining in the coils at the end of dialysis was flushed back into the patient using the routine wash-back technique already described. The wash-back fluid used was normal saline, the volume varying between 600 and 750 ml. The average amount of blood lost in the Chron-a-coils, 115 ml, was significantly greater ($P<0.01$) than the average loss in Ultra-flo 100s, 31 ml. The results are shown in Table 4.

Seven Chron-a-coils and 10 Ultra-flo 100 coils were collected under routine wash-back conditions and the blood loss measured by the radio-iron method (method 3). These results are also shown in Table 4. The average amount of blood lost in the Chron-a-coils was again significantly greater ($P<0.01$) than the average loss in the Ultra-flo 100s. The average losses by this method were not significantly different from those obtained using the radio-chromium method (Chron-a-coil $P>0.1$, Ultra-flo 100 $P>0.3$). Blood losses from a further 45 Ultra-flo 100 coils were determined under varying wash-back conditions and the results are shown in Table 5. No decrease in blood loss was effected by using a normal saline wash-back volume of 1000 ml at a pressure

TABLE 4

COIL BLOOD LOSSES AFTER ROUTINE WASH-BACK MEASURED BY THE RADIO-CHROMIUM AND RADIO-IRON METHOD

Chron-A-Coil	^{51}Cr	^{59}Fe
Number of coils	8	7
Mean loss (ml)	115	85
Standard error	12	20
	(P>0.1)	
Ultra-Flo 100	^{51}Cr	^{59}Fe
Number of coils	3	10
Mean loss (ml)	31	36
Standard error	9	6
	(P>0.3)	

Mean loss in CHRON-A-COILS by both methods was significantly greater (P<0.01)than mean loss in ULTRA-FLO 100S.

T A B L E 5

BLOOD LOSSES IN ULTRA-FLO 100 COILS

MEASURED BY THE RADIO-IRON METHOD

Coils	Wash-Back Conditions				
	Normal saline		5% Dextrose		Normal saline
	A	B	A	B	C
Number	15	10	10	5	5
Mean Blood Loss (ml)	23	36	14	11	23
Standard Error	5	6	4	3	9

Comparison of Means: Normal saline A vs B P>0.1

5 % Dextrose A vs B P>0.1

All normal saline vs all 5% dextrose P<0.02

A = 500 ml fluid at 0mm pressure

B = 600-750 ml fluid at 0mm pressure

C = 1000 ml fluid at 150mm pressure

of 150 mm compared with 500 ml at 0 mm pressure ($P>0.1$). Blood losses were shown to be reduced significantly ($P<0.02$) when dextrose was used instead of saline for the wash-back. Finally, blood losses were determined in 3 Ultra-flo 100 coils which were not placed in a dependent position during the wash-back with 500 ml of normal saline at 0 mm pressure. The average amount of blood retained in the coil was 85 ± 12 ml which is significantly greater ($P<0.001$) than the average loss of 23 ml when the coils were raised.

DISCUSSION

A division in the literature occurs between Eschbach et al. (1967) who have demonstrated iron overload in patients on regular dialysis therapy and Shaldon (1966) who found that his patients were iron deficient. There are two sources of iron available to these patients, apart from dietary iron, which could result in overload, namely iron from repeated transfusion or uptake from the dialysing fluid (Maher et al., 1965). In a previous study (Lawson et al., 1968) it was shown that uptake of labelled iron from the dialysate varied between 0.33 and 0.40% of the bath content of iron. The amount of iron absorbed in this way is clearly dependent upon the iron concentration of the tap water, but taking the maximum permissible concentration of iron in water, 500 µg per litre, a patient will only absorb 1 mg of iron per

week. In Glasgow, it would take 6 weeks of twice weekly dialysis for 1 mg of iron to be absorbed. Iron deficiency might arise if there was an iron loss from the patient into the dialysis fluid or if there were abnormal losses of blood. Lawson et al. (1968) found that the loss of injected iron-59 from a patient into the dialysing fluid was so low, 0.12% of the administered dose, that its significance was doubtful. It could be said, however, that insignificant quantities of iron were lost by this route.

By the criteria of Eschbach et al. (1970), 8 of the patients in Table 3 were iron deficient with a total iron binding capacity greater than 300 μg per 100 ml and a transferrin saturation of 18% or less, 2 of the patients were in normal iron balance with a total iron binding capacity of below 350 μg per 100 ml and a transferrin saturation of between 18 and 50% and 1 patient was in iron overload with a transferrin saturation of greater than 50%. The iron-59 absorption studies showed diminished oral iron uptake by these patients, the mean absorption being significantly less than in normal subjects. The mean percentage red cell iron-59 incorporation was also significantly less than in normals. There was no difference however between the oral absorption and the red cell incorporation of iron in these patients and in patients with chronic renal failure not on dialysis. The reduction in the oral absorption and red

cell incorporation of iron is likely to be the result of decreased erythropoiesis which has been shown to be a major factor in their control (Wheby,1966). Eschbach et al. (1970) found changes in the absorption of iron in chronic renal failure according to the iron status of the individual patient. A state of normal iron balance and of iron overload resulted in a mean absorption after a 5 mg dose of elemental iron of 3.5% and 3.6% respectively. A mean absorption of 58%, however, was found by these workers in a group of patients with iron deficiency. In this present study only 3 patients were iron deficient, no. 6 in Table 1 and nos. 4 and 10 in Table 3 , but no increased absorption of iron was apparent.

The mean long-term iron-59 excretion rates were significantly greater in the present patients than in those with chronic renal failure not on dialysis and in normal controls, there being an increase in turnover by at least a factor of 4. From these findings it would appear that patients on regular dialysis therapy are losing iron at a much faster rate than normal. Since it has been shown that insignificant quantities of iron were lost from the patient into the dialysing fluid, it would seem that the loss probably results from blood left in the coil at the end of dialysis together with blood removed for haematological investigations. Because of uncertainty in the red cell iron incorporation, it is difficult to make a

directly quantitative estimate of the blood loss. It is possible that the incorporation, which itself will be affected by the blood loss, might increase beyond 10 days because of the depressed rate of erythropoiesis in these patients.

The attempts to assess coil blood loss by the first method produced similar results to others (Evans et al. 1967, Muth 1969, Patel et al. 1967), but too small to account for some of the excretion rates observed. The accuracy of such a technique, however, is questionable. To mix adequately a few millilitres of red cells, even if the cells are lysed, in 1 litre of wash-out fluid and then obtain a representative sample for further measurement is extremely difficult. The measurement of the packed cell volume of the solution using a microhaematocrit has an accuracy of ± 80 to 90% and the measurement of the haemoglobin content has an accuracy of ± 30 to 40%. Consequently, considerable uncertainties are to be expected in the final answer and for this reason no further work was done using this method. The second method, using chromium-51, was initially chosen because of the ease of labelling the red cells. The results were variable and showed a high average coil blood loss. The losses, however, are in good agreement with the high excretion rates found in these patients. A source of error in the chromium method was the possible elution of chromium from the red cells following the labelling process (Mollinson,

1967) and it is known that free chromium can be selectively taken up by the dialysing membrane (Maher et al., 1965). Although it seemed unlikely that this introduced a significant error, the blood loss measurements were repeated using the radio-iron method. The iron-59 is incorporated into the red blood cells and hence any possible error due to elution is eliminated. Another possible source of error in the chromium method lay in the counting technique since variable sedimentation of the blood could occur within the coil. The determination of blood loss using the iron-59 method, however, depended upon comparison of counts from the same coil before and after an additional wash-out and the blood washed out was counted in an identical geometry to the standard. It is evident that the reservations about the chromium method are not well justified since the coil blood losses measured by these two methods were in good agreement. The results show that the blood losses can be significantly reduced by using Ultra-flo coils rather than Chron-a-coils and by raising the coil from its container and placing it on its side during the wash-back. A further reduction was seen when 5% dextrose was used as wash-back fluid in place of normal saline but this advantage may be offset by the unsuitability of dextrose if re-use of the coils is contemplated. This reduction in blood loss, which was certainly statistically significant ($P < 0.02$), is possibly

due to the wash-back with dextrose being more effective as a result of a decrease in clot formation. It has been shown that heparin inhibits the polymerization of the fibrin monomer to a polymer less effectively in the presence of saline (Triantaphyllopoulos, 1970). There was no significant reduction in blood loss when the wash-back volume was increased to 1000 ml, although, from clinical experience, the second 500 ml of a 1000 ml wash-back would still contain some blood. As well as an increase in volume, however, there was a change in the wash-back pressure and it would appear that the increased wash-back volume might have been offset by the higher flushing pressure.

The coil blood losses are therefore large enough to explain almost entirely the high rate of loss of iron-59 from the body seen in patients on regular dialysis therapy. These losses may be large enough to lead to iron deficiency but can be minimised by the method described. Since iron uptake from the dialysate is very low, iron overload in dialysed patients with chronic renal failure probably results from the repeated transfusion of blood. To maintain patients for long periods without transfusion, thus eliminating the potential danger of iron overload, it is necessary to minimise the amount of blood left in the coil at the end of dialysis and the amount of blood required for routine haematology.

SUMMARY

Iron metabolism has been investigated in patients with chronic renal failure. The oral absorption and the red cell incorporation of iron-59 in patients with chronic renal failure not on dialysis were significantly less than in normal controls, but the iron turnover rate was within normal limits. The anaemia of chronic renal impairment results from the depression of erythropoiesis, there being no evidence to suggest that iron deficiency plays a significant part in its aetiology.

The oral absorption and red cell incorporation of iron-59 in patients with chronic renal failure on regular dialysis therapy were similar to that of patients not requiring dialysis and significantly less than in normal controls due to decreased erythropoiesis. The iron turnover rate, however, was considerably higher than in patients with chronic renal failure not on dialysis.

Blood losses in the Kolff twin-coil artificial kidney have been studied by radioisotope techniques using chromium-51 and iron-59 and the results obtained compared with the simpler haemoglobin and microhaematocrit methods. It has been shown that the true blood losses are much larger than those obtained by the simpler methods and that these losses almost entirely explain the high excretion rate of iron-59 seen in patients on regular dialysis therapy. If routine blood transfusion is to

be avoided, thus eliminating the potential danger of iron overload and reducing the risk of serum hepatitis, it is necessary to minimise all sources of blood loss. Blood left in the coil at the end of dialysis is the main source of loss in these patients and can be minimised by using Ultra-flo 100 coils rather than Chron-a-coils, by altering the position of the coil during the wash-back procedure and by varying the composition and pressure of the wash-back fluid. The advantage of using dextrose as a wash-back fluid is offset by its unsuitability if re-sterilization and hence re-use of the coil is intended.

CHAPTER VIII

THE MEASUREMENT OF TOTAL BODY POTASSIUM: CALIBRATION AND ERRORS

INTRODUCTION

Potassium is an essential body electrolyte which can be measured 'in vivo' by the detection of the naturally occuring radioisotope, potassium - 40, using a high sensitivity whole body monitor. Potassium - 40 emits gamma rays with an energy of 1.46 MeV and the observed whole body counting-rate can be related to the total body content of potassium.

The principle sources of uncertainty in measuring the absolute total body content of potassium are counting statistics, variation of the subject counting-rate per gram of potassium due to differences in body habitus, technical errors associated with reproducibility of the counting geometry and stability of the counting-rates of standards and background. Although these uncertainties are common to all types of whole body counters, their magnitude and relative importance will depend on the monitoring geometry and on the monitoring procedure. These factors have been investigated for a scanning-bed geometry in contrast to the tilting-chair or multiple detector stretcher geometries which are more commonly used.

The variation of the counting-rate per gram of potassium with body build has been examined by the administration of the artificial isotope potassium - 42, which emits a single gamma ray almost identical in energy, 1.52 MeV, to that of the natural potassium - 40. It attains a distribution in the body similar to that of native potassium (Burch and Spiers, 1953) and produces an instrument response which can be used to allow for self-absorption of the radiation and for body geometry. By comparison of the counting-rates of potassium - 40 and potassium - 42 in human subjects and in identical phantoms, the variation of the counting-rates with body build can be examined and a more precise estimate of the body content of potassium can be obtained (Miller 1962, Delwaide et al. 1963, Lorimer et al. 1965, Wilson 1964, Hughes and Williams 1967, Burkinshaw 1967). It was also of practical importance to derive a calibration factor, related to body parameters, which could be used to convert the result of a single potassium - 40 measurement on a subject to total body potassium without reference to potassium - 42. The probable error in estimating body potassium by this procedure was then compared with that used in other counting geometries.

MATERIALS AND METHODS

Thirty nine male and 30 female subjects were included in the calibration study. All were healthy volunteers or were suffering

from diseases which are not known to affect the distribution of potassium in the body.

The detector-face to bed distance was 30 cm and the scanning speed was 8.2 cm per minute for potassium - 40 and 40.8 cm per minute for potassium - 42. The background was counted for 20 minutes before and after each potassium - 40 measurement. Following the potassium - 40 scan, each subject was given an oral dose of 9 - 11 μCi potassium - 42. Approximately 24 hours post-administration, a further whole body measurement was made. The abdominal and chest sections of a polythene phantom man (Bush, 1946) were filled with 1000g of potassium as potassium chloride and the complete phantom was scanned several times during each series of measurements. The phantom was remeasured with about 2.5 μCi potassium - 42 similarly distributed, this activity being typical of the amount in the subject at 24 hours after administration.

The procedure for dispensing and assaying the potassium - 42 underwent some changes as experience showed where errors might appear. In the final procedure, potassium - 42 chloride solution from the Radiochemical Centre, Amersham, was suitably diluted and dispensed by weight into polythene containers. The gamma ray output from these was then compared, using a reference ionisation chamber connected to a vibrating reed electrometer and recorder. Patients drank the doses from the containers

through a straw, followed by the rinsings. One dose was poured into the phantom, again with the rinsings. The residual activity in the containers and straws was measured with scintillation counter. This amount varied from zero to 0.05% of the dose. Comparison between the doses of potassium - 42 given to the patients and that used in the phantom was made from the weights of the solutions, corrected for the measured residual activity. The ion chamber measurements were used as a check. In general, agreement between the two estimations was within 2%. Urine was collected for the 24 hour period between administration of the potassium - 42 and whole body counting. A 10 ml aliquot was assayed in an M6 liquid sample G.M. counter. A standard for comparison was prepared from another dose, diluted with potassium chloride solution to minimise absorption of the radio-isotope onto glassware. The activity to be subtracted from the administered dose to give the amount remaining at 24 hours ranged from about 1% to 5% with a mean of 2.9%. In a few cases a check was also made on faecal radioactivity. This was found to be negligible.

To examine the precision of the calibration and monitoring procedures, replicate measurements were made in 8 of the subjects. The reproducibility of patient monitoring was studied in an additional subject by making 10 measurements of the potassium - 40 counting-rate in one week. In 2 other subjects the potassium - 40

counting-rate was measured at intervals over approximately 2 years. The reproducibility of monitoring potassium - 40 in the phantom was examined using the data obtained from routine measurements during a period of 5 months when body potassium measurements were being made. Confirmatory studies of reproducibility were undertaken with iron - 59 and cobalt - 58 in the same sections of an identical phantom. Variation is routine background counting-rates was also examined.

Total body potassium was estimated in 5 further subjects. The potassium - 40 counting-rate obtained in each case was expressed as grams of potassium using the calibration factors obtained from the earlier part of the study. For comparison, these subjects were also examined in the Leeds whole body monitor (Burkinshaw, 1967) and their body potassium independently estimated.

RESULTS

Typical counting-rates and the constancy of the background counting-rates are summarised in Table 1. The energy bands used for phantoms and human subjects were 1.36 - 1.56 MeV for potassium - 40 and 1.38 - 1.71 MeV for potassium - 42. A Compton band of 0.77 - 1.27 MeV was also used for both isotopes to check that there was no interference from other radioisotopes which might have been administered previously. Since counting statistics

TABLE 1

TYPICAL COUNTING-RATES AND CONSTANCY OF THE BACKGROUND FOR BODY POTASSIUM MEASUREMENTS

Potassium - 40: 0.973 cpm / gK

Potassium - 42: 2,000 cpm / μCi ^{42}K

CENTRE OR HOSPITAL	No. OF MEASUREMENTS	PERIOD OF TIME	MEAN BGD (cpm)	STD DEVIATION (cpm)	
				TOTAL	EXCESS *
WESTERN INFIRMARY, GLASGOW	18	21. 2.68-7. 7.69	116.7	2.57	0.89
SOUTHERN GENERAL, GLASGOW	31	11. 3.68-25. 7.68	111.2	2.57	1.02
WESTERN GENERAL, EDINBURGH	7	5.12.67-14.12.67	122.5	2.42	-
ROYAL INFIRMARY, EDINBURGH	28	14. 7.69-24. 7.69	116.0	2.38	-
S.R.R.C., EAST KILBRIDE	18	5. 8.68-4. 8.69	119.5	2.48	0.44

* The excess standard deviation was calculated by assuming:

$(\text{Total S.D.})^2 = (\text{Statistical S.D.})^2 + (\text{Excess S.D.})^2$.

In no case was the excess S.D. significantly different from the statistical S.D. ($P > 0.2$).

were inferior to those in the photopeak range, the results from the Compton band were not used in calculating the body potassium.

Total body potassium was calculated from the equation:

$$Kg = \frac{\text{subject } {}^{40}\text{K cpm}}{\text{phantom } {}^{40}\text{K cpm/gK}} \times \frac{\text{cpm/}\mu\text{Ci } {}^{42}\text{K phantom}}{\text{cpm/}\mu\text{Ci } {}^{42}\text{K subject}}$$

Variation of the counting-rate with body build was examined using the ratio:

$$F = \frac{\text{cpm/}\mu\text{Ci } {}^{42}\text{K phantom}}{\text{cpm/}\mu\text{Ci } {}^{42}\text{K subject}}$$

Table 2 summarises the results. Regression equations were computed by the method of least squares relating the F values to the weight and height of the subjects. The equations were computed for F with weight (W) + height (H), with (W/H), (W/H^2), (W/H^3) and $\sqrt{W/H}$. For the sexes individually and combined the multiple regressions of F on W + H were clearly the best fit and, as the equations obtained for males and females separately were not significantly different, the single equation $F = 0.0914 + 3.323 \times 10^{-3}W(kg) + 4.782 \times 10^{-3}H(cm)$ for the combined sexes can be used. The standard deviation from regression of the predicted F value for a subject of 74.8 kg in weight and 168 cm in height is 3.3%.

The overall error in measuring total body potassium includes counting statistics, errors in the positioning of phantoms and

TABLE 2

TOTAL BODY POTASSIUM TOGETHER WITH OTHER RELEVANT DATA

Case	Age (Yrs)	Wt (kg)	Ht (cm)	F Value*	Total K (g)
MALES					
1	51	61.2	169.1	1.068	128.9
2	29	78.0	177.8	1.149	135.1
3	28	74.8	167.6	1.128	138.0
4	44	69.9	166.4	1.148	115.0
5	35	57.2	161.3	1.108	127.8
6	59	76.2	170.8	1.205	132.8
7	38	84.8	180.3	1.287	158.6
8	23	70.0	172.7	1.105	134.9
9	23	72.0	172.7	1.169	160.1
10	29	71.0	167.6	1.147	111.3
11	35	73.0	179.1	1.217	130.3
12	60	84.0	171.5	1.299	156.0
13	63	70.8	173.0	1.135	100.5
14	34	55.7	172.1	1.124	130.0
15	56	78.5	173.4	1.229	139.1
16	49	66.5	166.1	1.142	118.9
17	58	61.6	177.8	1.190	100.2
18	26	70.0	172.7	1.059	140.4
19	55	50.8	163.2	1.006	104.7
20	56	57.1	165.1	1.010	110.9
21	54	87.1	189.2	1.257	164.0
22	49	74.0	188.6	1.244	138.0
23	48	49.9	153.7	1.022	89.5
24	35	73.0	179.1	1.151	145.2
25	24	76.7	170.2	1.167	145.3
26	27	68.7	180.3	1.184	137.1
27	27	68.7	180.3	1.177	134.9
28	54	86.4	189.2	1.246	166.5
29	54	86.4	189.2	1.282	165.5
30	27	61.7	169.5	1.133	146.6
31	27	61.7	169.5	1.119	147.2
32	52	55.6	167.6	1.047	99.7
33	28	68.0	179.1	1.172	148.7
34	28	68.0	179.1	1.200	150.9
35	61	54.4	167.6	1.029	95.6
36	45	68.0	170.2	1.096	148.0
37	27	82.6	185.4	1.252	161.9
38	24	67.1	177.2	1.164	151.7
39	64	71.2	169.5	1.141	132.9

*See Text

TABLE 2

(CONTINUED)

Case	Age (Yrs)	Wt (kg)	Ht (cm)	F Value*	Total K (g)
FEMALES					
1	43	49.9	170.2	1.051	86.7
2	24	99.8	162.0	1.198	98.7
3	63	78.0	158.4	1.148	81.3
4	30	61.5	161.3	1.022	87.4
5	68	98.4	158.0	1.208	89.4
6	29	93.4	154.5	1.119	103.8
7	33	40.0	144.8	0.915	63.2
8	43	62.1	160.0	1.103	102.2
9	53	44.0	157.5	0.999	64.8
10	32	39.5	152.4	1.023	76.3
11	43	69.4	170.2	1.194	95.3
12	36	57.0	163.8	1.022	72.5
13	52	85.1	157.5	1.062	93.0
14	58	83.4	158.8	1.067	98.8
15	26	42.2	147.3	0.886	66.5
16	48	50.2	152.4	0.995	79.5
17	46	50.8	158.8	0.986	78.2
18	58	63.5	148.6	1.029	78.6
19	51	61.2	161.3	1.036	96.2
20	58	82.6	157.5	1.123	103.2
21	58	82.6	157.5	1.126	105.1
22	26	52.2	157.5	1.013	90.6
23	26	52.2	157.5	1.009	92.9
24	57	84.6	151.8	1.122	92.1
25	41	87.3	158.1	1.119	97.0
26	41	87.3	158.1	1.085	92.7
27	61	40.8	154.3	0.981	75.9
28	60	52.2	174.0	1.119	94.6
29	44	74.8	170.8	1.179	101.3
30	64	49.0	151.1	0.971	69.9

* See text

subject, constancy of the background and the technical errors in estimating the F value. The statistical standard deviation in measuring potassium - 40 was 0.6% for the phantom and 2.3% for a subject with 140 gK. The corresponding value for potassium - 42 was 0.5% for both the phantom and the subject. Repetitive potassium - 40 measurements with the phantom containing 1000 gK and with subject 9 (Table 3) gave an observed standard deviation which was not significantly greater than the statistical standard deviation. It can therefore be concluded that positioning errors in both phantom and subject made negligible contributions to the observed standard deviation. A similar conclusion was obtained with the radioisotopes in the phantom. The variation in background counting-rates was analysed during each week of the calibration study. The standard deviations obtained were not significantly greater than the statistical standard deviations. As the background counting-rate was measured before and after each subject, errors due to extraneous changes in background should be negligible or comparatively small. The technical errors in estimating the F value include, together with potassium - 42 counting statistics, errors in dispensing the potassium - 42, in collection and assay of excreta and in estimating the residues. An analysis of these errors suggests that the standard deviation associated with these procedures is about 1%, this being similar to that estimated by Hughes and Williams (1967). The standard

TABLE 3

RESULTS OF REPLICATE POTASSIUM - 40 MEASUREMENTS

CASE No.	No. OF MEASUREMENTS	PERIOD OF MEASUREMENT	TOTAL BODY K(g)	MEAN gK	S.D. ± gK
WITH ESTIMATED F VALUE					
1	3	6 months	164.0 166.5 165.5 -	165.3	1.3
2	3	9 months	99.0 103.0 105.1 -	102.4	3.1
3	2	1 week	137.1 134.9 - -	136.0	1.6
4	2	1 week	146.6 147.2 - -	146.9	1.3
5	2	1 week	148.7 150.9 - -	149.8	1.6
6	2	1 week	90.6 92.9 - -	91.8	1.6
7	2	1 week	92.7 97.0 - -	94.9	3.0
8	2	1 week	124.5 121.9 - -	123.2	1.8
WITH PREDICTED F VALUE					
9	10	1 week	RANGE: 129.4 - 138.4	132.7	2.8
10	4	22 months	162.2 162.9 158.6 171.5	163.8	5.5
11	4	8 months	81.0 85.8 91.6 89.4	87.0	4.6

deviation in measuring body potassium by the administration of potassium - 42 to an individual is obtained by combining the statistical standard deviations of the phantoms containing potassium - 40 (0.6%) and potassium - 42 (0.5%), of the subject's potassium - 40 (2.3% for 140 gK) and potassium - 42 (0.5%) and the standard deviation associated with the potassium - 42 dispensing and other procedures (1.0%). The combined standard deviation is 2.7%.

The standard deviation from regression has two components: (i) technical errors in the measurement of F and (ii) deviations of the true values of F from the regression (Burkinshaw, 1967). The technical errors of measurement were summed and subtracted from the standard deviation from regression (3.3%). The corrected standard deviation of the predicted F was then 3.06%. The standard deviation from regression was much greater than the instrumental error and presumably reflected variations in body build. When the F value is predicted from the regression line, the standard deviation in measuring body potassium is obtained by combining the standard deviation of prediction (3.06 %) with the statistical standard deviation of the phantom containing potassium - 40 (0.6%) and of the subject's potassium - 40 (2.3% for 140 gK). The combined standard deviation is 3.9%.

The results of replicate measurements of body potassium in human subjects are summarised in Table 3. In subjects 1 to 8,

the potassium - 42 calibration procedure was repeated and in subjects 9 to 11 body potassium was estimated from the potassium -40 counting-rate and the F value predicted from the derived relationship.

Body potassium estimates obtained in the five subjects measured both in the present counter and in the plastic scintillator tilting-chair whole body counter at Leeds are summarised in Table 4. The results agree within the errors estimated for both monitors.

DISCUSSION

It has been shown (Boddy 1967, King 1967) that the counting statistics in measuring total body potassium in the human subject were as good as other high sensitivity monitors in Britain with the exception of the steel room monitor at Leeds Infirmary, particularly that using the large plastic scintillation detectors. There are, however, additional to the counting statistics, other sources of error in calibrating whole body monitors to take account of variation in the potassium - 40 counting-rate with the habitus of individuals. The present procedure followed closely that of other workers comparing the counting-rates of potassium - 40 and administered potassium - 42 in human subjects with those from corresponding measurements in a phantom. The standard deviation of this method is obtained by combining the statistical

TABLE 4

TOTAL BODY POTASSIUM MEASURED IN MERLIN AND LEEDS INFIRMARY WHOLE BODY MONITOR

SUBJECT	TOTAL BODY POTASSIUM (gK ± 1 6gK)*	
	MERLIN	LEEDS
MALES		
1	162.2 ± 6.1	162.2 ± 6.6
2	130.2 ± 5.4	126.1 ± 5.2
3	131.5 ± 5.4	129.4 ± 5.4
FEMALES		
4	81.0 ± 4.2	86.2 ± 3.6
5	104.7 ± 4.8	107.0 ± 4.4

* See Text

standard deviations with the technical errors in the positioning of the subject and phantom and with the errors in potassium - 42 dispensing and associated procedures. In this study, the latter errors were comparatively small and from the data given, the standard deviation in measuring a subject with 140 gK by this method was 2.7%. This is similar to that estimated for other geometries. For the plastic scintillator chair-geometry and the multiple NaI detector stretcher geometry, Burkinshaw (1967) gave the corresponding standard deviation as 3.2% in both geometries. Barnaby and Jasani (1968) estimated a value of 3.4% for a large area liquid scintillation counter.

When the F value is predicted from the weight and height of the patient, these being readily and accurately obtainable, only a single whole body measurement is required, administration of a radioactive isotope is avoided and an estimate of the body potassium is provided more rapidly. However, the standard deviation of the potassium estimate is now greater, 3.9%, since it must include uncertainties associated with the prediction of F. This value can also be compared with those for other monitors derived in a similar manner. From measurements on 25 subjects, Burkinshaw (1967) found that the corresponding values for the plastic scintillator chair and the NaI stretcher geometries were 3.6% and 2.8% respectively. Contrary to the present findings, the error for the NaI stretcher geometry is less than the error

estimated for the calibration of individual subjects. A standard deviation of about 3.8% was found in 14 subjects by Barnaby and Jasani (1968) with a large area liquid scintillation counter which is less than that of 5% estimated both by Oberhausen (1966) with a 2π liquid scintillation counter and by Delwaide (1969) with a 4π plastifluor monitor. There is a significant discrepancy between the calibration data for the single detector tilting-chair geometries. Miller (1962) found that the potassium - 40 counting-rate was virtually independent of body build and applying a constant calibration factor to 23 subjects, estimated that the standard deviation was 2.7%. Lorimer et al. (1965) and Joyet and Baudraz (1968), however, found that correlation of the calibration factor with body habitus was necessary and even then obtained standard deviations of about 5%.

It is evident that the present monitoring geometry and calibration procedure compare favourably with others whether the calibration factor is derived from the administration of potassium-42 or is predicted from the regression line. In these measurements, factors such as counter stability and drift, reproducibility of positioning and variations in counting-rate with body build may contribute more to the total standard deviation than the counting statistics. Burkinshaw (1967), by direct comparison of a plastic scintillator chair geometry with an adjacent multiple NaI detector stretcher geometry, estimated a similar standard deviation

in both geometries after the administration of potassium - 42. When predicting the calibration factor, however, the standard deviation was lower in the stretcher geometry. Even when sequential measurements are made in the same subject, and the errors of calibration are avoided, the standard deviations are usually significantly greater than the statistical standard deviations because of the remaining factors (Burkinshaw 1967, Delwaide 1969). In the present monitor, the remaining factors do not contribute significantly to the total standard deviation, 2.4% for sequential measurements on a subject with 140 gK.

The comparative measurements of body potassium in five subjects (Table 4) were made with monitors of quite different geometries and using apparently dissimilar regression lines for predicting the calibration factors. Excellent agreement between the two monitors was obtained and the estimated errors were similar.

The present study and others, including those to which reference has been made, emphasise that the measurement of body potassium is not simple and is subject to a variety of errors, the relative importance of which differ with the monitoring geometry but all of which must be considered in evaluating the performance of a whole body monitor.

SUMMARY

Total body potassium has been measured in 39 male subjects and 30 female subjects using the potassium - 42 calibration technique. For a subject with 140 gK the total standard deviation was 2.7%. A relationship was derived enabling the calibration factor to be predicted from a subject's weight and height and the total standard deviation associated with this measurement for a subject of 75 kg in weight and 168 cm in height with 140 gK was estimated as 3.9%.

The precision of the calibration and monitoring procedures were examined in detail with respect to technical errors associated with reproducibility and the stability of the background counting-rates. Replicate measurements of the total body potassium were made with both the calculated calbration factors and those predicted from the regression line. It was evident that the monitoring geometry and calibration procedure compared favourably with that of other monitors.

Total body potassium was measured in 5 subjects in this present monitor and also, for comparison, in the Leeds whole body monitor. Good agreement between the estimates of the two monitors was obtained.

CHAPTER IX

THE PREDICTION OF TOTAL BODY POTASSIUM FROM WEIGHT, HEIGHT AND AGE

INTRODUCTION

It is well recognised that indirect measurements, such as serum potassium or exchangeable potassium, are a poor reflection of the total body content of this mineral. In acute experimental depletion of potassium, the serum potassium level may show a relationship to the extent of the depletion (Black, 1953). In clinical practice, however, this relationship is poor (Moore et al. 1954, 1955, Flear et al. 1957, Hughes et al. 1967, Surveyor and Hughes 1968). The measurement of exchangeable potassium using potassium - 42 is subject to mixing errors, the total exchangeable potassium being estimated as varying from 92 to 98% of the total body potassium (Remenchik and Miller 1962, Talso et al. 1960). Since a period of between 24 and 48 hours is required for the equilibration of the injected isotope with the natural body potassium, the procedure is inconvenient and the difficulties in collection and assay of excreta add to the intrinsic errors of the technique (Blainey et al., 1954). A direct measurement of total body potassium can only be made using a high sensitivity whole body monitor. When the monitor has been calibrated, no radioisotope

need be administered to the patient and the total body potassium is obtained rapidly, only a single measurement being required.

In general, the measurement of total body potassium is of clinical value only if the normal for the particular subject is known. When studying changes in body potassium due to varying clinical conditions or to treatment, the patient acts as his own control and a normal value for a particular patient is not required. Strictly, it is not even necessary to express total body potassium in absolute terms such as milliequivalents or grams. However, in certain clinical disorders it is of considerable significance to know if the total body potassium is different from that in the normal and to what extent. In order to predict the normal level, it is necessary to express the total body potassium in terms of body weight or of lean body mass which can be derived from total body water. Total body water can be measured by an isotope dilution technique using tritiated water or antipyrine. The clinical value of this technique is limited since it involves an equilibration time of 3 to 6 hours together with the collection and assay of either serial blood or urine samples. Recently, however,it has been shown that total body water and by extrapolation lean body mass can be readily predicted from weight and height (Hume, 1966). Since total body water and total body potassium are closely related (Moore et al., 1963), it seemed of interest to determine how well total body potassium could be

predicted from weight and height (Boddy, King, Hume and Weyers 1970).

MATERIALS AND METHODS

Total body potassium has been measured in 103 subjects, comprising 49 males and 54 females. All were healthy members of the Reactor Centre staff or of the hospital medical and nursing staff and were on a normal diet and fuild intake at the time of the study. The age of the subjects ranged from 18 years to 77 years with a mean of 35 years in males and 36 years in females. The counting-rate in the potassium - 40 photopeak (1.36 - 1.56 MeV) was converted to grams or milliequivalents of potassium using calibration data obtained previously (see Chapter VIII).

The total body water (T.B.W.) and lean body mass (L.B.M.) were calculated from the weight (W) and height (H) of each subject according to the formula of Hume (1966) in a revised form (Hume and Weyers, 1970). The relationships are as follows:

MALES: $T.B.W. = 0.2968\ W\ (kg) + 0.1948\ H\ (cm) - 14.0129$

FEMALES: $T.B.W. = 0.1838\ W\ (kg) + 0.3446\ H\ (cm) - 35.2701$

where $L.B.M. = T.B.W. \times \frac{100}{73}$ kg (Pace and Rathbun, 1945.)

RESULTS

The total body potassium (T.B.K.) together with revelant physiological data for each subject are summarised in Table 1. Total potassium per kilogram predicted lean body mass is also given for each subject.

The linear reqressions of total body potassium in grams on weight, height and predicted lean body, with males and females treated separately and as one group, were calculated by the method of least squares. Age was then included as an additional variable and the regressions were recomputed. It was found that the male and female groups had to be treated separately since, when they were combined, the lines were significantly different. Where appropriate, the statistical significance of additional variables was examined by a sequential F - test. The results are summarised in Table 2. There was a highly significant correlation between total body potassium and the predicted lean body mass which was improved when age was included in the analysis. The standard deviation from regression was 7.5% of the mean K for males and 7.7% of the mean K for females. Significant correlations were obtained with weight and height separately which were then improved by a multiple regression of weight, height and age. The standard deviation from regression was 7.3% for males and 7.7% for females.

Similar regression equations were calculated of total body potassium in grams per kilogram body wt (gK/kg B.Wt) on age,

TABLE 1

TOTAL BODY POTASSIUM TOGETHER WITH RELEVANT PHYSIOLOGICAL DATA

SUBJECT	AGE	WT (kg)	HT (cms)	L.B.M. (kg)	gK	gK/kg L.B.M.
MALES						
1	54	70.0	156.2	51.0	114.5	2.25
2	20	57.0	171.5	49.7	119.8	2.41
3	24	72.8	177.8	57.9	167.4	2.89
4	30	53.0	166.4	46.8	109.2	2.34
5	30	75.0	174.0	57.7	148.9	2.58
6	44	54.0	170.2	48.2	116.3	2.42
7	30	62.0	177.8	53.5	152.2	2.85
8	34	74.0	167.6	55.6	149.6	2.69
9	26	64.0	181.6	55.3	165.3	2.99
10	26	56.7	161.9	47.1	107.8	2.29
11	58	55.3	171.5	49.1	111.5	2.27
12	25	68.0	172.7	54.5	144.8	2.66
13	25	80.7	175.3	60.4	169.9	2.81
14	24	58.6	179.1	52.4	140.5	2.68
15	24	60.9	174.0	52.0	144.8	2.79
16	24	68.0	179.1	56.2	143.1	2.54
17	24	69.0	180.3	57.0	163.3	2.87
18	25	83.5	184.2	63.9	187.5	2.93
19	24	78.9	170.2	58.3	152.6	2.62
20	43	59.0	157.5	46.8	97.7	2.09
21	51	61.7	160.7	48.8	104.8	2.15

TABLE 1

SUBJECT	AGE	WT (kg)	HT (cms)	L.B.M. (kg)	gK	gK/kg L.B.M.
MALES						
22	46	67.6	170.2	53.7	136.0	2.53
23	43	78.5	174.0	59.2	146.5	2.48
24	22	52.0	170.8	47.5	129.6	2.73
25	77	47.2	157.5	42.0	90.7	2.16
26	31	80.7	175.5	60.5	167.1	2.77
27	35	74.5	177.8	58.5	134.6	2.30
28	35	70.5	176.5	56.6	131.6	2.33
29	27	69.4	171.5	54.8	120.1	2.19
30	64	73.9	170.2	56.3	124.1	2.21
31	58	54.4	151.1	43.2	88.1	2.04
32	65	62.1	171.5	51.8	107.8	2.08
33	43	81.7	177.8	61.5	158.3	2.58
34	47	71.7	177.8	57.4	135.3	2.36
35	43	75.3	184.2	60.6	160.6	2.65
36	43	80.7	172.7	59.7	131.9	2.21
37	42	60.5	170.2	50.8	124.0	2.44
38	25	69.5	177.8	56.7	129.5	2.28
39	28	72.7	182.9	59.3	153.3	2.59
40	26	65.9	177.8	55.1	156.9	2.85
41	38	61.8	167.6	50.9	126.4	2.48
42	24	60.0	172.7	51.3	131.7	2.57
43	23	85.4	191.8	66.5	172.7	2.60
44	46	92.3	171.5	63.6	143.4	2.26
45	26	65.4	174.0	53.7	130.3	2.43
46	21	63.5	177.8	54.1	151.7	2.80
47	20	69.9	175.3	56.0	162.0	2.89

TABLE 1

SUBJECT	AGE	WT (kg)	HT (cms)	L.B.M. (kg)	gK	gK/kg L.B.M.
MALES						
48	21	78.0	181.6	61.0	172.9	2.83
49	22	65.3	167.6	51.3	130.8	2.55
MEAN	35	68.0	173.2	54.6	138.1	2.52
FEMALES						
50	23	62.4	169.0	47.2	107.2	2.27
51	29	57.0	161.3	42.2	100.2	2.38
52	23	54.0	163.8	42.6	111.4	2.62
53	23	45.4	156.2	36.9	86.9	2.36
54	24	60.8	160.0	42.5	100.2	2.36
55	22	69.8	166.4	47.8	106.0	2.22
56	23	60.0	161.3	42.9	101.4	2.36
57	25	50.8	165.1	42.4	104.5	2.46
58	70	54.4	154.9	38.5	78.0	2.03
59	38	63.0	160.0	43.1	95.6	2.22
60	23	44.1	152.4	34.7	80.7	2.32
61	25	49.4	158.8	39.1	83.9	2.15
62	23	60.3	168.9	46.6	105.0	2.25
63	23	69.9	172.7	50.8	125.2	2.46
64	23	51.0	157.5	38.9	91.9	2.36
65	23	52.2	162.6	41.6	90.3	2.17
66	53	48.1	157.5	38.1	83.0	2.18
67	24	48.5	165.1	41.8	96.7	2.31
68	23	53.1	154.9	38.2	97.1	2.54
69	24	53.5	161.3	41.3	85.5	2.07
70	24	64.9	167.6	47.1	102.6	2.18
71	20	64.0	162.6	44.6	120.6	2.71
72	37	60.8	165.1	44.9	97.3	2.17

TABLE 1

SUBJECT	AGE	WT (kg)	HT (cms)	L.B.M. (kg)	gK	gK/kg L.B.M.
FEMALES						
73	29	62.8	165.1	45.4	92.3	2.03
74	24	88.0	168.9	53.6	108.6	2.03
75	42	62.1	165.1	45.3	89.0	1.97
76	24	52.6	163.8	42.3	78.9	1.87
77	25	76.7	182.9	57.3	134.6	2.35
78	59	57.0	157.5	40.4	81.5	2.02
79	38	61.2	161.3	43.2	96.3	2.23
80	21	55.4	160.0	41.2	99.9	2.43
81	58	56.3	151.1	37.2	77.1	2.07
82	42	65.3	147.3	37.7	90.0	2.39
83	62	65.3	157.5	42.5	86.1	2.03
84	48	49.9	162.6	41.0	80.4	1.96
85	41	50.3	157.5	38.7	81.4	2.10
86	60	54.0	153.7	37.8	81.6	2.16
87	49	68.5	156.2	42.7	93.1	2.18
88	57	66.2	172.7	49.9	96.8	1.94
89	60	64.0	161.3	43.9	81.3	1.85
90	60	62.6	172.7	49.0	90.5	1.85
91	65	64.0	166.4	46.4	96.1	2.07
92	68	62.1	156.2	41.1	79.5	1.94
93	72	47.2	160.0	39.1	78.9	2.02
94	49	43.1	148.6	32.7	78.4	2.40
95	21	62.8	168.9	47.2	107.2	2.27
96	34	55.0	167.6	44.8	101.7	2.27
97	18	49.5	163.8	41.7	103.7	2.49
98	24	61.7	171.5	48.2	116.6	2.42

TABLE 1

SUBJECT	AGE	WT (kg)	HT (cms)	L.B.M. (kg)	gK	gK/kg L.B.M.
FEMALES						
99	19	48.5	156.2	37.6	94.8	2.52
100	18	57.2	160.0	42.0	89.5	2.13
101	28	69.9	171.5	50.2	131.3	2.62
102	41	73.5	171.5	51.1	123.8	2.54
103	30	61.7	167.6	45.6	99.8	2.19
MEAN	36	58.7	162.4	43.2	96.2	2.23

TABLE 2

CORRELATION OF TOTAL BODY POTASSIUM (gK) WITH WEIGHT (W), HEIGHT (H), LEAN BODY MASS (L.B.M.) AND AGE

INDEPENDENT VARIABLES	CORRELATION COEFFICIENT		STD DEV'N FROM REGRESSION (% MEAN K)	
	MALES	FEMALES	MALES	FEMALES
W	0.688	0.594	12.3	11.9
H	0.818	0.732	9.7	10.1
W and H	0.875	0.765	8.3	9.6
W,H and Age	0.907	0.857	7.3	7.8
LBM	0.830	0.761	9.4	9.6
LBM and Age	0.897	0.856	7.5	7.7

weight / height^3 and also weight / height^2, weight / height and height^3 / weight with age. The results are summarised in Table 3. A mean value and standard deviation of 2.04 ± 0.25 gK/kg B.Wt was obtained for male subjects and 1.65 ± 0.21 gK/kg B.Wt for females. The correlation of gK/kg B.Wt with age gave a standard deviation from regression of about 10%. This was reduced when factors involving weight and height were also included.

Analagous regression equations were computed with gK/kg L.B.M. The mean value and standard deviation of gK/kg L.B.M. was 2.52 ± 0.26 in male subjects and 2.23 ± 0.21 in female subjects. The standard deviation from regression of gK/kg L.B.M. on age was about 7.5% and was not significantly improved by additional factors involving weight and height (Table 4).

The equations selected, using weight, height and age, were as follows:

MALES: gK = 0.9369W (kg) + 1.3744H (cm) - 0.4728 Age - 147.0903

FEMALES: gK = 0.5773W (kg) + 0.8628H (cm) - 0.3540 Age - 65.2602

The fit with lean body mass and with other relationships of weight and height were apparently as good.

DISCUSSION

Since, in a normal male and female population, the total body

TABLE 3

CORRELATION OF TOTAL BODY POTASSIUM PER KILOGRAM BODY WEIGHT (gK/kg B.W.) WITH AGE AND RATIOS OF WEIGHT (W) AND HEIGHT (H)

INDEPENDENT VARIABLES	CORRELATION COEFFICIENT		STD DEV'N FROM REGRESSION (% MEAN gK/kg B.W.)	
	MALES	FEMALES	MALES	FEMALES
None +			12.3	12.7
Age	0.613	0.606	10.0	10.4
W/H^3	0.721	0.679	8.7	9.6
W/H^3 and Age	0.826	0.805	7.2	7.8
W/H^2 and Age	0.801	0.829	7.6	7.4
W/H and Age	0.747	0.809	8.6	7.7
H^3/W and Age	0.840	0.814	6.9	7.7

+ Mean gK/kg B.W. = 2.04 males, 1.65 females

TABLE 4

CORRELATION OF TOTAL BODY POTASSIUM PER KILOGRAM LEAN BODY MASS (gK/kg L.B.M.) WITH AGE AND RATIOS OF WEIGHT (W) AND HEIGHT (H)

INDEPENDENT VARIABLES	CORRELATION COEFFICIENT		STD DEV'N FROM REGRESSION (% MEAN gK/kg L.B.M.)	
	MALES	FEMALES	MALES	FEMALES
None +			10.3	9.4
Age	0.684	0.608	7.6	7.5
W/H^3	0.348	0.110	9.8	9.4
W/H^3 and Age	0.697	0.612	7.6	7.5
H^3/W	0.346	0.114	9.8	9.4
H^3/W and Age	0.699	0.612	7.5	7.5

+ Mean gK/kg L.B.M. = 2.52 Males, 2.23 females

potassium content varies greatly with changes in body habitus and with age (Table 1) the total body potassium expressed as an absolute figure has only limited worth. For this reason, in order to define a "normal" value for body potassium, it has in the past been related to other body measurements, most commonly body weight. It is well established from cadaver studies (Shohl 1939, Widdowson et al. 1951, Forbes and Lewis 1956) that body potassium is most closely related to the lean body mass or fat-free body weight. The lean body mass of an individual, however, can only be derived indirectly from complicated body density studies (Steinkamp et al. 1965, Durnin and Rahaman 1967) or from the measurement of total body water which is constantly related to lean body mass (Pace and Rathbun, 1945). Recently, however, it was shown that total body water and hence lean body mass can be predicted with reasonable accuracy from height and weight (Hume, 1966). It seemed of importance, therefore, to relate total body potassium to such readily obtainable data as weight, height, age and predicted lean body mass.

The correlation with lean body mass and age gave a standard deviation from regression of about 7.6% which was not materially improved when weight, height and age were the unrestricted independent variables (Table 2). Similar standard deviations from regression were obtained when gK/kg B.Wt was correlated with age and various ratios of weight and height (Table 3) and

when gK/kg L.B.M. was correlated with age (Table 4). In the latter correlation, the inclusion of various ratios of weight and height as additional variables made no material improvement. The normal total body potassium in a given individual can therefore be predicted with a standard deviation of 7.6% using only the weight, height and age of the subject.

At Leeds Infirmary, colleagues Hughes et al. (1967) and Williams et al. (1967) related total body potassium to lean body mass derived from the measurement of total body water. The standard deviations from regression obtained were 6.4% of the mean in male subjects and 9.4% in female subjects which are scarcely better than those obtained in the present study using a predicted value for total body water and hence lean body mass. The mean values of 2.52 ± 0.26 gK/kg L.B.M. in males and 2.23 ± 0.21 gK/kg L.B.M. in females were similar to those obtained by Hughes and colleagues (1967) of 2.98 ± 0.20 gK/kg L.B.M. in males and, in an earlier study, by Allen et al. (1960) of 2.56 and 2.28 gK/kg L.B.M. in males and females respectively with standard deviations of about 5.6%. Although these standard deviations are apparently better than those found both in the present study and by Hughes and colleagues (1967), a contributary factor might be that only one male and female subject exceeded 42 years of age. The present age range was from 20 - 77 years in males and from 18 - 72 years in females and in the study by Hughes et al. (1967)

was from 31 - 75 years in males.

In other studies, exchangeable body potassium (K_E) has been correlated with lean body mass. Corsa et al. (1950) obtained a mean and standard deviation of 55.4 ± 4.1 m Eq K_E/kg L.B.M. from similtaneous measurements of exchangeable potassium and total body water in a group of young adult males. In a similarly homogeneous group of male students, corresponding values of 62.5 ± 2.9 m Eq K_E/kg L.B.M. were obtained, while in normal male and female patients figures of 59.7 ± 8.5 m Eq K_E/kg L.B.M. and 62.2 ± 9.4 m Eq K_E/kg L.B.M. were found respectively (Talso et al., 1960). The results of Talso et al. (1960) support the earlier suggestion that the age range of the subjects influence the standard deviation of the estimated normal body potassium. The smallest standard deviation was obtained in the student group with an age range of 23 - 38 years. In the normal patients the age ranges were 14 - 75 and 25 - 82 years in males and females respectively. In the young age groups, the smaller standard deviation may reflect an inevitably more mesomorphic group with less physiological variation.

Several investigators have related normal total body potassium and exchangeable potassium to one body parameter only, that of weight. When age was included in the analysis of total body potassium, standard deviations of between 11 and 19% were obtained (Anderson and Langham 1959, Meneely et al. 1962, Oberhausen and Onstead 1965). In the analysis of exchangeable potassium and body

weight, standard deviations of between 9 and 12% were obtained (Moore et al. 1954, Corsa et al. 1950, Blainey et al. 1954 and Talso et al. 1960).

Nicholson and Zilva (1964) analysed body constituents per kilogram body weight on the basis of a "leaness index" (H^3/W) or its reciprocal, and "obesity index" (W/H^3) and found that they could be used to predict normal values of body constituents over a wide range of body build. It was suggested that these indices gave the best correlation coefficients in measurements involving body fat (Fletcher, 1962). Fletcher (1962) and Edwards and Whyte (1962) related the estimated body fat to these ratios and also to the ratios W/H^2 and W/H. For comparison, the present results (gK/kg B.Wt)were correlated with these ratios together with age (Table 3) and a high degree of correlation was obtained. The findings, however, do not suggest a clear cut advantage in favour of either of the indices in normal subjects, only marginally lower standard deviations from regression being obtained with H^3/W and age in males and with W/H^2 and age in females.

Total body potassium per kilogram lean body mass was also correlated with age and ratios of weight and height (Table 4). The improvement in the standard deviation from regression is most marked with age alone whereas the changes with other relationships of weight, height and age are less consistent.

Finally, the findings have been compared with the results of

cadaver analysis. As indicated by Widdowson et al. (1951), few bodies available for such analyses would be strictly normal and the results require cautious interpretation if they are to be used as the basis of normality. With this reservation, in adult bodies thought to be free from any serious relevant disease and in normal electrolyte balance, a value of 66.8 m Eq K/kg L.B.M. was obtained by Shohl (1939), a value of 72.8 m Eq K/kg L.B.M. by Widdowson et al. (1951) and values of 66.5 and 66.6 m Eq K/kg L.B.M. by Forbes and Lewis (1956). These are in reasonable agreement with those obtained in this present study of 64.6 and 57.2 m Eq K/ kg L.B.M. in males and females respectively.

SUMMARY

In clinical practice, the measurement of total body potassium may only be meaningful if the "normal" for a particular subject is known. Total body potassium has been measured in 103 normal subjects and the values obtained correlated with lean body mass predicted from the weight and height of the individual and with various relationships of weight and height. Age was then included in the analysis as an additional variable. It was shown that the normal total body potassium can be predicted with a standard deviation from regression of about 7.5% using only the weight, height and age of the subject.

CHAPTER X

THE MEASUREMENT OF BODY POTASSIUM IN CLINICAL DISORDERS

INTRODUCTION

Abnormal body potassium levels may be associated with various diseases, resulting either from the disease itself, or, indirectly, from the effect of drug therapy. In order to illustrate the application of the techniques described in the previous two chapters, the total body potassium status has been investigated in three different clinical conditions.

It has been known for many years that electrolyte imbalance may occur in patients with renal impairment (Peters et al. 1929, Platt 1950, Schoch 1951), and this was the first condition to be studied, total body potassium being measured in patients with chronic renal failure and also in those receiving regular dialysis therapy. Although serum changes during haemodialysis have been studied extensively (Holmes et al. 1958 , Comty et al. 1964, Seedat 1968), little information is available on the effect of acute or chronic dialysis on whole body electrolytes.

The second clinical condition in which potassium status has been investigated is rheumatoid arthritis, where the effect of treatment with depot tetracosactrin was studied. Tetracosactrin

is a synthetic polypeptide and its duration of action has been prolonged beyond that of natural corticotrophin (ACTH) by complexing the active polypeptide with zinc phosphate as depot tetracosactrin. In the treatment of rheumatoid arthritis with a moderate dosage of oral corticosteroids or ACTH, an increase in the urinary excretion of potassium may occur (Copeman 1953), but does not usually result in hypokalaemia or require the administration of potassium supplement (West 1962, Savage et al. 1962). Preliminary trials using depot tetracosactrin have shown a consistent and highly significant fall in serum potassium and an increase in urinary excretion of potassium (Nuki et al. 1970).

Finally, total body potassium has been measured in a group of patients following bilateral ureterocolic anastomosis as a method of urinary diversion. The development of hypokalaemia following ureterocolic transplantation was first noted by Foster et al. (1950). Hyperchloraemic acidosis is a clinical condition with which chronic potassium deficiency may be associated (Reimer et al. 1951, Clarke et al. 1955) and is a recognised complication in this group of patients (Stamey 1956, Jacobs and Stirling 1962). The hypothesis is that the acidosis is primary and that the potassium deficiency ofter seen in these patients can be obviated by adequate treatment of the acidosis.

MATERIALS AND METHODS

Using the calibration factors derived previously (Chapter VIII), the subject counting-rate from the potassium-40 photopeak was expressed as grams of potassium without the administration of a radioisotope. From the weight, height and age of each patient, a predicted normal value for total body potassium was calculated (Chapter IX). For the different clinical disorders studied, the measured total body potassium values were compared with the predicted values using a paired Student's t test. For each individual patient, the measured value was compared with the predicted value as follows:

$$t = \frac{|K_P - K_A|}{SEE}$$

where K_P is the predicted value

K_A is the actual value

SEE is the standard error of the estimate.

The standard error of the estimate was calculated for each patient from the standard deviation from multiple regression:

$$SEE = S\sqrt{1 + 1/N + C_{11}(\bar{x}_1 - X_1)^2 + C_{22}(\bar{x}_2 - X_2)^2 + C_{33}(\bar{x}_3 - X_3)^2 + 2C_{12}(\bar{x}_1 - X_1)(\bar{x}_2 - X_2) + 2C_{13}(\bar{x}_1 - X_1)(\bar{x}_3 - X_3) + 2C_{23}(\bar{x}_2 - X_2)(\bar{x}_3 - X_3)}$$

where S is the standard deviation from regression

C the values of the inverse sums of squares and products matrix

$\bar{x}_1$, $\bar{x}_2$, $\bar{x}_3$ are the mean independent variables

and x_1, x_2, x_3 are the values of x for an individual patient.

The degrees of freedom for this significance test are (N - 4) where N is the number of results from which the regression equations were calculated (see Chapter IX).

Total body potassium was measured in 25 patients with chronic renal failure and in a further 32 patients who were also receiving regular dialysis therapy. Fourteen of the patients were undergoing chronic haemodialysis by means of a Kiil dialyser, each of them dialysed for two 14-hour periods per week. The potassium concentration in the dialysate fluid was 3.0 mEq/litre and the flow rate was 500 ml/min. The remaining 18 patients were undergoing haemodialysis using a Kolff twin-coil artificial kidney, each of them being dialysed for two 10-hour periods per week. The total dialysate volume was approximately 150 litres for each dialysis with a potassium concentration of 1.0 mEq/litre. Ultra-flo 100 coils were used. Eleven of the patients being dialysed on the Kiil artificial kidney were monitored immediately before and after dialysis in order to measure any changes in the total body potassium.

Nine patients with severe, active rheumatoid arthritis

requiring some form of corticosteroid therapy were admitted simultaneously to hospital for one month. The patients were all on a fixed dietary potassium intake of 65 mEq/24 hours for approximately 3 days before baseline measurements of plasma, urinary and total body potassium were made. The patients remained on this diet throughout the period of study. These investigations were repeated 14 days after commencing intramuscular therapy with 0.5 mg of depot tetracosactrin on alternate days and then again after a further 14 days of treatment during which time the patients were also receiving a potassium supplement (Slow-K, 1.2g tid) or a potassium retaining diuretic (Spironolactone, 50mg tid). The mean daily faecal excretion of potassium was measured in one patient, 3 day faecal collections being made during each week of the study. One patient did not finish the course of treatment and therefore the complete data are given for only 8 subjects.

Total body potassium was measured in 11 patients with bilateral ureterosigmoidal transplantation. In all patients, this was performed at least six months prior to the potassium study.

At the time of measurement all patients were active, ambulant and with the exception of the patients with rheumatoid arthritis, were taking a normal diet with free fluid intake. No patient was measured during an episode of acute electrolyte

imbalance or, again with the exception of the patients with rheumatoid arthritis, received any potassium supplement during the period of study.

RESULTS

The total body potassium and other relevant data in patients with chronic renal failure not on regular dialysis therapy are summarised in Table 1. A paired Student's t test showed the difference between the measured total body potassium and the predicted values not to be significant ($t = 0.62$, $P \gg 0.05$). However, testing the patients individually, the total body potassium was found to be low in cases 3, 4, 12 and 22 and to be high in cases 10, 13 and 15 ($P < 0.05$). The results in patients with chronic renal failure who were also receiving maintenance haemodialysis using a Kiil dialyser are summarised in Table 2a and using a Kolff twin-coil dialyser in Table 2b. The difference between the measured and predicted total body potassium was not significant in the first group ($t = 1.65$, $P > 0.05$) but was significant in the second ($t = 3.19$, $0.01 > P > 0.001$). Cases 2 and 5 in Table 2a and cases 11, 14, 15 and 18 in Table 2b were found to be depleted ($P < 0.05$). Total body potassium levels immediately before and after dialysis on the Kiil artificial kidney are given in Table 3. There was no significant change in the body potassium, either treating the groups as a whole

TABLE 1

TOTAL BODY POTASSIUM AND OTHER RELEVANT DATA

IN PATIENTS WITH CHRONIC RENAL FAILURE NOT ON DIALYSIS

CASE	ACE	HT (cm)	WT (kg)	LBM (kg)	MEASURED		PREDICTED	
					gK	gK/kg LBM	gK	gK/kgLBM
MALES								
1	49	170.2	74.1	56.3	125.1	2.22	133.0	2.36
2	49	162.6	73.5	49.8	131.8	2.65	122.0	2.45
3	54	176.5	77.0	55.7	117.7	2.11	142.0	2.55
4	33	176.5	68.6	52.8	112.8	2.14	144.1	2.73
5	37	177.8	76.8	56.0	154.9	2.77	151.7	2.71
6	27	157.5	54.8	41.9	100.4	2.40	107.9	2.58
7	44	163.8	58.6	45.2	125.1	2.77	121.5	2.69
8	16	158.8	51.5	41.2	110.5	2.68	111.8	2.71
9	43	175.3	63.5	50.8	128.1	2.52	132.9	2.62
10	34	171.5	79.4	54.7	171.4	3.13	146.9	2.69
11	47	179.1	81.2	57.9	139.0	2.40	152.9	2.64
12	75	176.5	80.7	56.8	112.1	1.97	135.6	2.39
FEMALES								
13	54	141.0	55.6	32.2	86.4	2.68	69.4	2.16
14	23	157.5	54.9	39.8	98.2	2.47	94.2	2.37
15	24	161.3	51.7	40.8	112.0	2.75	95.3	2.34
16	43	156.2	70.6	43.2	106.3	2.46	95.1	2.20
17	52	153.7	65.4	40.3	98.6	2.45	86.7	2.15
18	68	157.5	73.4	44.3	84.8	1.91	88.9	2.01
19	46	174.0	61.5	47.7	93.9	1.97	104.1	2.18
20	61	154.9	48.3	35.8	78.8	2.20	74.7	2.09
21	19	162.6	61.7	42.9	97.7	2.28	103.9	2.42
22	58	160.0	49.4	38.2	55.4	1.45	80.8	2.12
23	38	149.9	45.4	32.8	76.0	2.32	76.8	2.34
24	39	156.2	59.0	39.5	94.1	2.38	89.8	2.27
25	51	151.1	56.7	36.7	86.6	2.36	79.8	2.17

Compairing MEASURED gK with PREDICTED gK $t = 0.62$ $(P \gg 0.05)$

TABLE 2a

TOTAL BODY POTASSIUM AND OTHER RELEVANT DATA IN PATIENTS WITH CHRONIC RENAL FAILURE ON REGULAR DIALYSIS THERAPY

CASE	AGE	HT (cm)	WT (kg)	LBM(kg)	MEASURED		PREDICTED	
					gK	gK/kg LBM	gK	gK/kgLBM
MALES								
1	38	167.6	65.3	48.8	120.2	2.46	126.4	2.59
2	36	189.2	89.7	64.1	149.7	2.34	179.9	2.81
3	29	168.9	59.4	47.3	131.7	2.78	126.9	2.68
4	45	175.3	75.3	54.7	138.7	2.54	143.1	2.62
5	24	166.4	59.4	46.4	95.2	2.05	125.9	2.71
6	37	175.3	65.3	51.4	140.9	2.74	137.5	2.68
7	33	174.6	57.5	48.6	112.6	2.32	131.1	2.70
8	41	172.7	73.5	53.2	129.7	2.44	139.7	2.63
9	44	174.0	67.7	51.7	133.7	2.59	134.6	2.60
FEMALES								
10	42	156.2	50.3	36.9	88.7	2.40	83.7	2.27
11	30	152.4	45.4	33.9	71.3	2.10	81.8	2.41
12	43	165.1	53.5	41.6	108.7	2.61	92.9	2.23
13	23	151.1	50.3	34.8	85.1	2.45	86.0	2.47
14	35	153.7	45.8	34.5	83.6	2.42	81.4	2.36

Compairing MEASURED gK with PREDICTED gK $t = 1.65$ $(P > 0.05)$

TABLE 2b

CASE	AGE	HT (cm)	WT (kg)	LBM (kg)	MEASURED		PREDICTED	
					gK	gK/kg LBM	gK	gK/kgLBM
MALES								
1	39	166.4	69.4	53.4	111.2	2.08	128.1	2.40
2	29	177.8	64.4	54.4	141.6	2.60	143.8	2.64
3	43	182.9	65.3	56.2	140.3	2.50	145.1	2.58
4	41	170.2	55.1	48.6	114.5	2.36	119.0	2.45
5	39	162.6	52.8	45.7	104.3	2.28	107.4	2.35
6	23	167.6	56.3	48.4	118.9	2.46	125.1	2.58
7	27	170.2	59.4	50.4	138.1	2.74	129.7	2.57
8	26	177.8	60.8	53.0	132.4	2.50	141.9	2.68
9	34	167.6	52.8	47.0	123.9	2.64	116.6	2.48
10	40	161.3	54.7	46.1	120.4	2.61	106.9	2.32
11	33	177.8	57.6	49.7	115.3	2.32	135.6	2.73
12	26	177.8	68.5	53.3	139.0	2.61	149.1	2.80
13	40	172.7	61.7	49.3	113.1	2.29	129.1	2.62
14	19	163.8	42.4	40.0	76.9	1.92	108.7	2.72
FEMALES								
15	38	166.4	53.5	43.7	78.9	1.81	95.8	2.19
16	31	163.8	46.7	43.5	80.4	1.85	92.1	2.12
17	31	163.8	48.0	41.1	84.2	2.05	92.8	2.26
18	20	167.6	51.3	43.7	86.6	1.98	101.9	2.33

Compairing MEASURED gK with PREDICTED gK $t = 3.19$ $(0.01 > P > 0.001)$

TABLE 3

TOTAL BODY POTASSIUM BEFORE AND AFTER DIALYSIS

CASE	POTASSIUM (g)	
	BEFORE DIALYSIS	AFTER DIALYSIS
1	88.7	88.4
2	120.2	116.4
3	149.7	150.2
4	131.7	128.1
5	138.7	137.7
6	95.2	93.3
7	140.9	144.4
8	112.6	117.8
9	129.7	135.0
10	71.3	73.1
11	107.0	99.8

$t = 0.12 \quad (P \gg 0.05)$

($t = 0.12$, $P \gg 0.05$), or in the individual cases.

Table 4 summarises the results of total body potassium measurements together with other relevant data in patients with rheumatoid arthritis. A paired t test showed the measured total body potassium to be significantly less than the predicted values for these patients ($t = 2.63$, $0.05 > P > 0.02$). Testing the results individually, cases 2 and 9 were found to be depleted ($P < 0.05$). The faecal and urinary potassium levels during treatment with depot tetracosactrin are given in Table 5 and the total body potassium and plasma potassium in Tables 6 and 7 respectively. After both the first and second two weeks of treatment there was no significant change in the total body potassium ($P > 0.05$). However, the fall in the plasma potassium was highly significant after the first two weeks of treatment ($t = 6.36$, $P < 0.001$) and the levels did not change in the second two weeks of the study ($t = 1.88$, $P > 0.10$). The potassium balance in these patients during the period of treatment is shown in Figure 1. Assuming the faecal potassium loss in case 6 to be a realistic estimate of the loss in the group as a whole, good agreement (to within ± 5-8%) was obtained between the potassium intake and output. The study did not take place in a metabolic unit and this agreement indicates the degree of patient co-operation that was obtained.

Total body potassium together with other relevant data in

TABLE 4

TOTAL BODY POTASSIUM IN RHEUMATOID ARTHRITIS

CASE	AGE	HT (cm)	WT (kg)	LBM (kg)	MEASURED		PREDICTED	
					gK	gK/kg LBM	gK	gK/kg LBM
MALES								
1	57	167.6	52.8	44.7	96.0	2.15	105.7	2.36
FEMALES								
2	46	151.1	39.4	31.5	56.1	1.78	71.6	2.27
3	49	154.9	50.4	36.3	88.5	2.44	80.1	2.21
4	23	170.2	56.1	44.5	91.5	2.06	105.8	2.38
5	51	158.8	59.3	40.7	81.2	2.00	87.9	2.16
6	53	168.9	66.5	47.0	100.8	2.15	100.1	2.13
7	65	152.4	61.2	38.5	77.5	2.01	78.6	2.04
8	53	161.3	91.7	51.3	95.4	1.86	108.1	2.11
9	46	157.5	58.5	39.9	70.8	1.77	88.1	2.21

Compairing MEASURED gK with PREDICTED gK $t = 2.63$ $(0.05 > P > 0.02)$

TABLE 5

FAECAL AND URINARY POTASSIUM DURING TREATMENT WITH DEPOT TETRACOSACTRIN

WEEK OF TREATMENT	MEAN FAECAL K (mEq/day)	MEAN URINARY K (mEq/day)
1	14.8	46
2	10.7	
3	7.5	83 *
4	16.8	45 ǂ

* In 5 patients on Slow K

ǂ In 3 patients on Spironolactone

Mean faecal K measured in case 6 only

TABLE 6

TOTAL BODY POTASSIUM IN PATIENTS WITH RHEUMATOID ARTHRITIS BEFORE, DURING AND AFTER TREATMENT WITH DEPOT TETRACOSACTRIN

CASE	gK PRE-TREATMENT	gK AFTER 1ST 2 WEEKS TREATMENT	gK AFTER 2ND 2 WEEKS TREATMENT
	A	B	C
1 *	96.0	97.5	94.5
2 ǂ	56.1	56.4	56.6
3 *	88.5	85.1	87.7
4 *	91.5	91.8	92.6
5 *	81.2	81.0	87.8
6 *	100.8	92.9	104.4
7 ǂ	77.5	74.6	76.8
8 ǂ	95.4	87.2	85.0
MEAN	85.9	83.3	85.7

* Slow K (1.2g tid - 48 mEqK/day)

ǂ Spironolactone (50mg tid)

Compairing group A with B $t = 1.92$ $(P > 0.05)$

B with C $t = 1.40$ $(P > 0.10)$

A with C $t = 0.12$ $(P > 0.10)$

TABLE 7

PLASMA POTASSIUM BEFORE AND DURING

TREATMENT WITH DEPOT TETRACOSACTRIN

CASE	PRE-TREATMENT PLASMA K (mEq/l)	MEAN VALUE DURING 1st 2 WEEKS OF TREATMENT (mEq/l)	MEAN VALUE DURING 2nd 2 WEEKS OF TREATMENT (mEq/l)
	A	B	C
1 *	3.9	3.6	3.6
2 ǂ	3.7	2.8	2.6
3 *	3.8	3.3	3.5
4 *	3.9	3.6	3.4
5 *	3.8	3.3	3.9
6 *	3.6	3.3	3.7
7 ǂ	3.6	3.2	3.0
8 ǂ	3.9	3.2	3.2
MEAN	3.8	3.3	3.4

* Slow K (1.2g tid - 48 mEqK/day)

ǂ Spironolactone (50mg tid)

Compairing group A with B $t = 6.36$ $(P<0.001)$

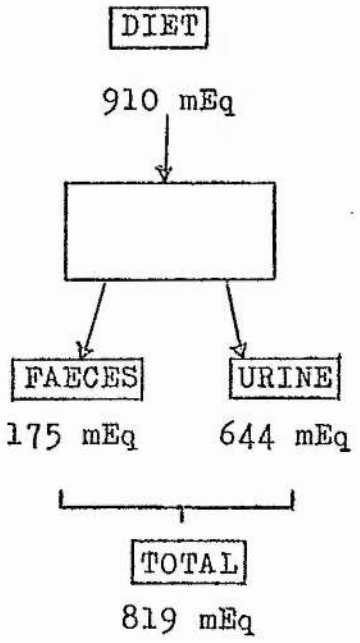
B with C $t = 1.88$ $(P>0.10)$

A with C $t = 2.87$ $(0.05>P>0.02)$

Fig. 1

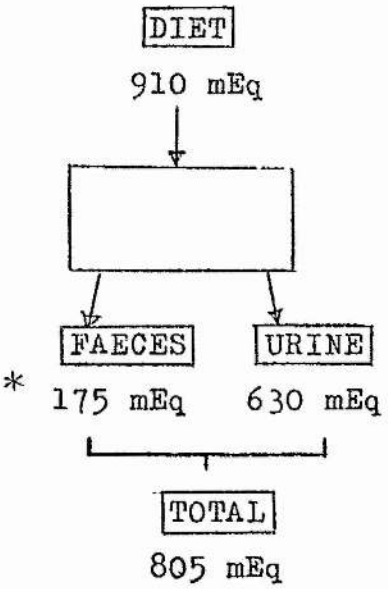
DIAGRAM OF POTASSIUM BALANCE DURING TREATMENT WITH DEPOT TETRACOSACTRIN

a) Balance during first 2 weeks

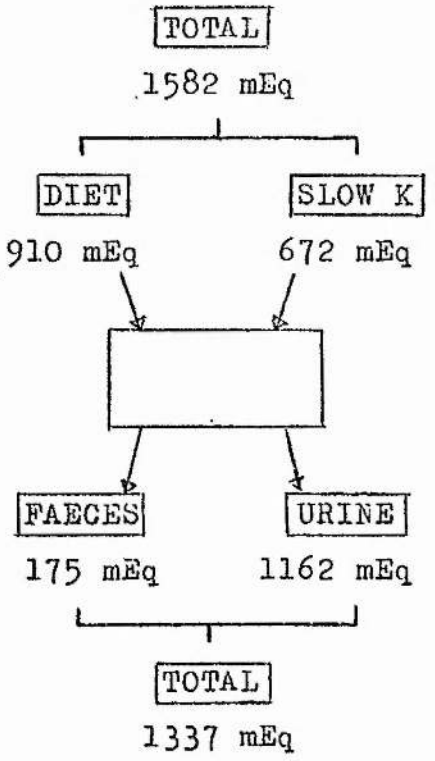


Balance during second 2 weeks

b) In patients receiving Spironolactone



c) In patients receiving Slow K



* Assuming Spironolactone does not effect faecal K levels

patients with colonic urinary diversions are given in Table 8. Although there was no significant difference between the measured and predicted total body potassium ($t = 0.57$, $P \gg 0.05$), testing the patients individually showed the potassium level to be low in cases 6 and 7 ($P = 0.05$) and to be high in cases 3, 4 and 5 ($P < 0.05$).

DISCUSSION

An evaluation of the potassium status of patients with chronic renal disease has depended on serum potassium, exchangeable potassium or muscle biopsy all of which are subject to considerable uncertainty when extrapolation is made to total potassium content. In the present study, whole body potassium was measured directly in a group of patients with chronic renal failure and compared with the expected values, the results were low in 16% of the subjects studied and were high in 12%. Moore et al. (1954) state that an elevation in the serum potassium concentration is a feature commonly associated with chronic renal disease and found that the exchangeable potassium was considerably lower than in healthy subjects even though a high serum potassium concentration coexists. However, Leaf and Camera (1949) and Berliner et al. (1950) found that hyperkalaemia is rarely a feature of chronic renal failure due to the ability of the diseased kidney to maintain potassium excretion as a result

TABLE 8

TOTAL BODY POTASSIUM AFTER URETEROSIGMOIDOSTOMY

CASE	AGE	HT (cm)	WT (kg)	LBM (kg)	MEASURED		PREDICTED	
					gK	gK/kg LBM	gK	gK/kg LBM
MALES								
1	52	162.6	46.2	40.8	105.1	2.58	95.0	2.33
2	53	174.7	66.4	51.5	143.8	2.79	130.1	2.53
3	66	157.5	52.5	41.1	104.4	2.54	87.3	2.12
4	63	154.9	39.6	36.0	95.0	2.64	73.1	2.03
5	56	156.2	65.9	45.1	125.0	2.77	102.8	2.28
6	63	171.5	74.6	53.1	109.0	2.05	128.7	2.42
7	54	174.0	87.5	58.2	124.6	2.14	148.5	2.55
8	64	174.0	80.3	55.8	144.2	2.58	137.0	2.46
9	72	174.0	88.0	58.4	128.1	2.19	140.4	2.40
10	60	168.9	71.4	51.2	123.5	2.41	123.5	2.41
FEMALE								
11	59	153.7	57.9	38.1	74.8	1.96	79.9	2.10

Compairing MEASURED gK with PREDICTED gK $t = 0.57$ $(P \gg 0.05)$

of tubular secretion. It is felt that such tubular secretion may contribute to the development of potassium deficiency in patients with advanced renal disease (Schoch, 1951). Furthermore, in these cases, it is possible that there may be enhanced faecal excretion of potassium (Hayes and Robinson, 1965). Graham et al. (1970) found the intracellular water and potassium content of skeletal muscle biopsy in patients with renal impairment remained almost normal except for the reduction of cell potassium content in the occasional patient. The variation in the present results would seem to corroborate the findings of these other workers. The results are being further examined to determine whether either the severity or the cause of the disease correlates with the potassium status of the patients.

The maintenance of potassium balance in patients on regular dialysis therapy depends on the frequency of dialysis and on the potassium concentration of the dialysate. Total body potassium in the group of patients being maintained by the Kiil dialyser was not significantly different from the predicted normal values, with only 2 of the individual patients being below these values. However, the difference between the measured and predicted total body potassium in the group being maintained by the Kolff twin-coil dialyser was significant and 4 of the individual patients were depleted. There was no significant change in the body potassium levels immediately before and after dialysis on the

Kiil artificial kidney and since the potassium balance is being maintained in this group of patients, the result was not unexpected. The minimum detectable change in body potassium would have been about ± 8% (2 6). Morgan et al. (1970) measured the total body potassium in 21 patients with chronic renal failure being treated on a Kiil dialyser with three 10 - hour periods of dialysis per week against a dialysate fluid containing 1.5 mEq K/litre and also found no evidence of potassium depletion. Seedat (1969) made a study of the effect of chronic haemodialysis on the exchangeable potassium in 7 patients being treated by means of a Kiil dialyser with a potassium concentration in the dialysate fluid of 1 mEq/litre. In 5 of the 7 patients studied there was a deficit in the exchangeable potassium of 20 to 35% and it was suggested that the level of 1 mEqK/litre in the dialysate may be too low. However, it should be noted that the exchangeable potassium is also low in patients with chronic renal failure who are not receiving regular dialysis therapy (Moore et al., 1954) so that it cannot be concluded unequivocally that the deficit in Seedat's patients is a result of dialysis. Total body potassium has been measured by Johny et al. (1970 A) in 15 patients on maintenance haemodialysis using a Kolff twin-coil artificial kidney with a dialysate potassium concentration of 1 mEq/litre. The patients were dialysed for 8 hours twice weekly and comparing the potassium per kg body weight with an

expected value for normal subjects, over half of the patients appeared to be depleted. However, insufficient information was given to determine the number of patients in which this conclusion was statistically justified. Apart from the wide range of normal variability in potassium in terms of body weight, counting statistics alone would result in an uncertainty of ± 4.5% for a subject with 140gK (Johny et al., 1970 B). Both Johnson et al. (1969) and Novak et al. (1969) found that if dialysis is limited to two 12 hour periods per week potassium depletion is not produced even if a potassium free dialysate is used. In this present study, however, and also in that of Johny et al. (1970 A), the group of patients receiving treatment on the Kolff twin-coil artificial kidney did become potassium depleted. The dialysate potassium concentration used in the different centres is variable indicating the lack of information concerning the ideal level and also the inadequate knowledge of potassium balance during haemodialysis. A further study is at present in progress using the Kolff artificial kidney to measure the direction and magnitude of potassium change during dialysis.

Preliminary trials in the treatment of rheumatoid arthritis with depot tetracosactrin have shown consistent and highly significant falls in plasma potassium which have even occurred when an oral supplement of potassium as Slow-K (1.2g tid) was administered (Nuki et al., 1970). Twenty-four hour urine studies

did seem to suggest that therapy with depot tetracosactrin is accompanied by an increased excretion of potassium. Furthermore, the falls in plasma potassium appeared to be largely unaccompanied by clinical symptoms. In this present study, total body potassium was measured in a group of patients with rheumatoid arthritis before commencing therapy with depot tetracosactrin and the measured total body potassium was significantly less than the predicted normal values, although this difference was significant in only two of the individual patients. The results are in agreement with those of LaCelle et al. (1969) who found weight related total body potassium to be lower in patients with rheumatoid arthritis than in controls. Throughout the course of treatment, there was no significant change in the total body potassium, confirming the results of the balance study. However, significant falls in the plasma potassium levels occurred during the first two weeks of treatment and these levels did not recover with the administration of either Slow-K or Spironolactone. The difference in the total body potassium and plasma potassium illustrates their poor relationship in clinical practice. The administration of large doses of corticosteroids often results in a fall in the plasma potassium concentration with the absence of any increased loss of potassium from the body (Bagshawe et al. 1965, Ross and Hurst 1965, LaCelle et al. 1969). Treatment with depot tetracosactrin seems to

result in a similar effect in patients with rheumatoid arthritis, not leading to a further depletion of potassium, but probably producing a shift of potassium into the cells.

Total body potassium in patients following ureterosigmoid anastomoses did not differ significantly from the predicted normal values, although when the cases were tested individually the potassium level was low in two of the patients and was high in three. Williams et al. (1967) measured body potassium in a comparable group comprising 27 patients of whom 23 had ureterocolic anastomoses and 4 had rectosigmoid bladders. Eighteen of the patients showed deficits of between 9 and 30%. Within this ureterocolic group, over half of the patients showed some acidosis and most of these had hyperchloraemia. In another study, the exchangeable potassium was measured in 12 patients following ureterosigmoid anastomoses by Ansell et al. (1961) who found only 1 patient within the normal range and an average deficit in the remainder of about 33%. All the patients were slightly acidotic and all were hyperchloraemic except for 1 depleted patient. The gross potassium depletion seen by these two groups was absent in this present study. The total body potassium was low in only 2 of the patients and the difference between the measured and predicted potassium values was only just significant at the 5% confidence limit. Williams et al. (1967) studied the potassium content of patients following

ureteroileal anastomoses and in over 30% of this group found an excess of whole body potassium. This tendency to potassium retention was also seen in 3 of the present patients. Williams and colleagues suggested the excess of potassium may be related to renal function. Previous studies on the migration of ions in the small bowel have shown that when the potassium concentration of a perfusing solution is more than three times the plasma concentration, then potassium absorption occurs (Pyrah et al. 1965). If the function of the kidneys is normal, homeostasis of the potassium can be achieved but there may be danger of potassium retention if renal function is impaired. The development of renal failure in these patients is not uncommon and can result from ascending infection due to faecal contamination. The results appear to confirm the original hypothesis that the potassium deficiency in these patients can be obviated, at least in part, by treatment of the hyperchloraemic acidosis. It should be emphasised that the patients were not receiving potassium supplement either during or prior to the study.

SUMMARY

To illustrate the application of the techniques described in the previous two chapters, total body potassium has been measured in three clinical disorders.

The first condition studied was chronic renal failure and

comparing the measured total body potassium with the patients' predicted value, the results were low in 16% of the subjects and high in 12%. This variation appears to corroborate the findings of other workers. In patients who were also receiving regular dialysis therapy, the difference between the measured and predicted total body potassium was not significant when a Kiil artificial kidney was used, with only 2 of the individual patients being below the predicted normal. However, the measured total body potassium was significantly less than the predicted values using a Kolff twin-coil dialyser and 4 of the individual patients were depleted. There was no change in the body potassium levels immediately before and after dialysis on the Kiil dialyser. A further study is at present in progress using the Kolff artifical kidney to measure potassium kinetics during dialysis.

The second clinical condition in which the potassium status has been investigated is rheumatoid arthritis, where the effect of treatment with depot tetracosactrin was studied. The pre-treatment potassium content of these patients was below the predicted normal value. Throughout the course of therapy, however, there was no detectable change in the total body potassium, although the fall in the plasma potassium was highly significant.

Finally, total body potassium has been measured in a group of patients following ureterosigmoid anastomoses. The difference between the measured and predicted total body potassium was not

significant but testing the patients individually showed the potassium level to be marginally low in 2 of the cases and to be high in 3. The results appear to confirm the original hypothesis that the metabolic sequelae of this type of urinary diversion which includes potassium depletion usually in association with hyperchloraemic acidosis can be obviated by adequate treatment of the acidosis.

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